Science and Medicine

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New hope Gene therapy for adrenoleukodystrophy

Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy

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Background: X-linked adrenoleukodystrophy (ALD) is a severe brain demyelinating disease in boys that is caused by a deficiency in ALD protein, an adenosine triphosphate-binding cassette transporter encoded by the ABCD1 gene. ALD progression can be halted by allogeneic hematopoietic cell transplantation (HCT).

Methods: The authors performed a gene therapy trial in 2 ALD patients for whom there were no matched donors. Autologous CD34+ cells were removed from the patients, genetically corrected ex vivo with a lentiviral vector encoding wild-type ABCD1, and then reinfused into the patients after they had received myeloablative treatment.

Results: Over a span of 24–30 months of follow-up, polyclonal reconstitution was detected, with 9–14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein. These results strongly suggested that hematopoietic stem cells were transduced in the patients. Beginning 14–16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the 2 patients stopped – a clinical outcome comparable to that achieved by allogeneic HCT.

Conclusion: Lentiviral-mediated gene therapy of hematopoietic stem cells can provide clinical benefits in ALD.

Although adrenoleukodystrophy often presents as an endocrine disorder with adrenal insufficiency and to a lesser extent Leydig cell insufficiency, the major impact of this devastating disease is on white matter demyelination. Approximately 50% of affected males present in mid-childhood with progressive demyelinating disease leading to death in adolescence. The group of Patrick Aubourg has been essential along the years in delineating the disease through establishment of allogeneic bone marrow transplantation as an efficient therapy, cloning the gene and refuting undue claims of dietary manipulation [1–3]. Quite interestingly, the favorable effect of allogeneic bone marrow transplantation demonstrated that replacement of deficient bone marrow-derived microglial cells was sufficient to cure the central nervous system involvement but had no effect on the adrenal dysfunction – an interesting observation for further research. However, bone marrow transplantation is a risky procedure and is often impeded by lack of familial or unrelated donor. A large collaborative group of pediatric neurologists and endocrinologists, basic scientists and clinical hematologists concurred to construct HIV-derived lentiviral vectors and transduce CD34+ hematopoietic stem cells from 2 patients, which were reinfused after myeloablation. The results, although preliminary, are quite striking since they show long-term engraftment of transduced cells, partial correction of the biochemical alterations and favorable clinical evolution. More importantly, no preferential clonal expansion was observed as compared to previously observed integration-related mutagenesis and leukemogenesis with retroviral vectors. This study is important not only for those affected or involved in the care of adrenoleukodystrophy, but also for the field of gene therapy which has been slowed down by several setbacks [4]. It confirms that a metabolic disease affecting the brain can be improved by hematopoietic stem cell correction and suggests that similar lentiviral approaches might be ben-

eficial to other genetic diseases. More importantly, it shows that persistence in clinical and basic study of a single disease can lead to major advances in medicine.

Origins and functional impact of copy number variation in the human genome

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Context: Structural variations of DNA >1 kb in size account for most bases that vary among human genomes, but are still relatively under-ascertained.

Methods: This study use tiling oligonucleotide microarrays, comprising 42 million probes, to generate a comprehensive map of 11,700 copy number variations (CNVs) >443 bp, of which most (8,599) have been validated independently. For 4,978 of these CNVs, they generated reference genotypes from 450 individuals of European, African or East-Asian ancestry.

Results: The predominant mutational mechanisms differ among CNV size classes. Retrotransposition has duplicated and inserted some coding and non-coding DNA segments randomly around the genome. Furthermore, by correlation with known trait-associated single nucleotide polymorphisms (SNPs), they identified 30 loci with CNVs that are candidates for influencing disease susceptibility.

Conclusions: Having assessed the completeness of this map and the patterns of linkage disequilibrium between CNVs and SNPs, this report concludes that, for complex traits, the heritability void left by genome-wide association studies will not be accounted for by common CNVs.

For type 2 diabetes, for example, there are now around 30 genetic variants known to influence susceptibility to the disease, but these only account for about 10% of the known inherited risk of developing these conditions. One theory for this so-called 'missing heritability' was that it may have been caused by copy number variations (CNVs). Yet, several studies of diabetes and other complex diseases found that commonly occurring CNV are unlikely to play a major role in such diseases. This study analyzed common CNVs in DNA samples from 3,000 healthy volunteers and compared them to 16,000 patients – 2,000 each with bipolar disorder, breast cancer, coronary artery disease, Crohn's disease, hypertension, rheumatoid arthritis, type 1 diabetes and type 2 diabetes. They identified and confirmed three loci with commonly occurring CNV, and all three had been identified previously by searching for SNPs, and none of the three CNV loci is believed to contribute to disease. It seems unlikely that common CNVs play a major role in the genetic basis of these or other common complex diseases, either through particular CNVs having a strong effect or through a large number of CNVs each contributing a small effect.

Endogenous non-retroviral RNA virus elements in mammalian genomes

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Nature 2010;463:84–87

Context: Retroviruses are the only group of viruses known to have left a fossil record, in the form of endogenous proviruses, and approximately 8% of the human genome is made up of these elements. Although many other viruses, including non-retroviral RNA viruses, are known to generate DNA forms of their own genomes during replication, none has been found as DNA in the germline of animals. Bornaviruses, a genus of non-segmented, negative-sense RNA virus, are unique among RNA viruses in that they establish persistent infection in the cell nucleus.

Results: Here they show that elements homologous to the nucleoprotein (N) gene of bornavirus exist in the genomes of several mammalian species, including humans, non-human primates, rodents and elephants. These sequences have been designated endogenous Borna-like N (EBLN) elements. Some of the primate EBLNs contain an intact open reading frame and are expressed as mRNA. Phylogenetic analyses showed that EBLNs seem to have been generated by different insertional events in each specific

animal family. Furthermore, the EBLN of a ground squirrel was formed by a recent integration event, whereas those in primates must have been formed more than 40 million years ago. They also show that the N mRNA of a current mammalian bornavirus, Borna disease virus, can form EBLN-like elements in the genomes of persistently infected cultured cells.

Conclusions: These results provide the first evidence for endogenization of non-retroviral virus-derived elements in mammalian genomes and give novel insights not only into generation of endogenous elements, but also into a role of bornavirus as a source of genetic novelty in its host.

This report provides evidence of endogenous sequences derived from a non-retroviral RNA virus in mammalian species. Phylogenetic analyses demonstrate that the oldest primate gene of bornavirus must have appeared in an ancestor of primates at least 40 million years, providing a new source of genetic innovation in their hosts. It is likely that these are processed pseudogenes derived from ancient bornavirus infections. Despite replication during tens of millions of years as exogenous viruses, the amino acid sequences of current Borna disease virus proteins seem surprisingly conserved. The paper also raises the possibility that, like some endogenous retroviruses, the Borna genes may have some function in their host species – us.

DNA methylation and gene expression differences in children conceived in vitro or in vivo

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Hum Mol Genet 2009;18:3769–3778

Context: Epidemiological data indicate that children conceived in vitro have a greater relative risk of low birth-weight, major and minor birth defects, and rare disorders involving imprinted genes, suggesting that epigenetic changes may be associated with assisted reproduction.

Methods: This study examined DNA methylation at more than 700 genes (1,536 CpG sites) in placenta and cord blood and measured gene expression levels of a subset of genes that differed in methylation levels between children conceived in vitro versus in vivo.

Results: The results suggest that in vitro conception is associated with lower mean methylation at CpG sites in placenta and higher mean methylation at CpG sites in cord blood. It also finds that in vitro conception-associated DNA methylation differences are associated with gene expression differences at both imprinted and non-imprinted genes. The range of inter-individual variation in gene expression of the in vitro and in vivo groups overlaps substantially but some individuals from the in vitro group differ from the in vivo group mean by more than two standard deviations. Several of the genes whose expression differs between the two groups have been implicated in chronic metabolic disorders, such as obesity and type 2 diabetes.

Conclusions: These findings suggest that there may be epigenetic differences in the gametes or early embryos derived from couples undergoing treatment for infertility. Alternatively, assisted reproduction technology may have an effect on global patterns of DNA methylation and gene expression. In either case, these differences or changes may affect long-term patterns of gene expression.

IVF now accounts for at least 1–2% of all live births in the Western world. Although the overall rate of congenital anomalies in children conceived by IVF is low (4–6%), this rate still represents a significant increase over the background rate of major malformations (3%). When indices of pre- and post-natal development are measured, IVF children, as a group, do not differ significantly from their control counterparts, except for having an increased incidence of low birth-weight and being slightly taller. This report analyzed DNA methylation at a large number of CpG sites in placenta and cord blood, comparing children conceived by IVF with a control group. They observed an overall lower level of specific CpG site methylation in placenta and higher level in cord blood. If these differences are characteristic of differences in embryonic versus extraembryonic tissues, it may suggest differences in the way ‘outer’ and ‘inner’ blastomeres of pre-implantation embryos respond to in vitro culture. These epigenetic differences have the potential to affect embryonic development and fetal growth, as well as to influence the long-term patterns of gene expression associated with the increased risk of many human diseases. The authors suggest that there is also a possibility that these changes could pass to the children of IVF babies and spread through the human gene pool.

Activating mutations in FGFR3 and HRAS reveal a shared genetic origin for congenital disorders and testicular tumors

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Context: Genes mutated in congenital malformation syndromes are frequently implicated in oncogenesis, but the causative germline and somatic mutations occur in separate cells at different times of an organism's life. Here we unify these processes to a single cellular event for mutations arising in male germ cells that show a paternal age effect.

Results: Screening of 30 spermatocytic seminomas for oncogenic mutations in 17 genes identified 2 mutations in FGFR3 (both 1948A>G, encoding K650E, which causes thanatophoric dysplasia in the germline) and 5 mutations in HRAS. Massively parallel sequencing of sperm DNA showed that levels of the FGFR3 mutation increase with paternal age and that the mutation spectrum at the Lys650 codon is similar to that observed in bladder cancer. Most spermatocytic seminomas show increased immunoreactivity for FGFR3 and/or HRAS.

Conclusions: This article proposes that paternal age-effect mutations activate a common 'selfish' pathway supporting proliferation in the testis, leading to diverse phenotypes in the next generation including fetal lethality, congenital syndromes and cancer predisposition.

This article offers a link between achondroplasia and Apert, Noonan and Costello syndromes and seminomas of older men: they all may arise from germ cell defects. FGFR3 and HRAS mutations induce gain of function, and develop as the testicle ages, and encourage the mutant cells to divide and multiply with copies of the mutation to each daughter cell. Hence, the number of sperm carrying this mutation increases as men get older, raising the risk to older fathers of having affected children. The findings link the processes of mutation in the soma (causing neoplasia) and germline (causing heritable disorders in the next generation), which normally occur in different cells, to a mutational event likely happening in the same cell. The authors call them 'selfish' because the mutations benefit the germ cell but are harmful to offspring. But other diseases seem to be more frequent in the offspring of older fathers, including breast cancer, autism and schizophrenia; the authors suggest that mutations might contribute to these diseases.

New mechanisms

All in the Aire – cytokine antibodies, mucocutaneous candidiasis and neonatal tolerance

Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I

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Background: Most patients with autoimmune polyendocrine syndrome type I (APS-I) display chronic mucocutaneous candidiasis.

Methods: The authors hypothesized that this chronic mucocutaneous candidiasis might result from autoimmunity to interleukin (IL)-17 cytokines.

Results: High titers of autoantibodies against IL-17A, IL-17F, and/or IL-22 were found in the sera of all 33 patients tested, as detected by multiplex particle-based flow cytometry. The auto-Abs against IL-17A, IL-17F, and IL-22 were specific in the 5 patients tested, as shown by Western blotting. The auto-Abs against IL-17A were neutralizing as shown by bioassays of IL-17A activity. None of the 37 healthy controls and none of the 103 patients with other autoimmune disorders tested had such autoantibodies. None of the patients with APS-I had auto-Abs against cytokines previously shown to cause other well-defined clinical syndromes in other patients (IL-6, interferon- γ , or granulocyte/macrophage colony-stimulating factor) or against other cytokines (IL-1 β , IL-10, IL-12, IL-18, IL-21, IL-23, IL-26, interferon- β , tumor necrosis factor- α , or transforming growth factor- β).

Conclusion: The findings suggest that autoantibodies against IL-17A, IL-17F, and IL-22 may cause chronic mucocutaneous candidiasis in patients with APS-I.

Neonatal tolerance revisited: a perinatal window for Aire control of autoimmunity

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Background: There has long been conceptual and experimental support for, but also challenges to, the notion that the initial period of the immune system's development is particularly important for the establishment of tolerance to self. The display of self antigens by thymic epithelial cells is key to inducing tolerance in the T-lymphocyte compartment, a process enhanced by the Aire transcription factor.

Methods: Doxycycline-regulated transgene to target Aire expression to the thymic epithelium, complementing the Aire knockout in a temporally controlled manner.

Results: Aire is essential in the perinatal period to prevent the multiorgan autoimmunity that is typical of Aire deficiency. Aire could be shut down soon thereafter and remain off for long periods, with few deleterious consequences. The lymphopenic state present in neonates was a factor in this dichotomy because inducing lymphopenia during Aire turnoff in adults recreated the disease, which, conversely, could be ameliorated by supplementing neonates with adult lymphocytes.

Conclusion: Aire expression during the perinatal period is both necessary and sufficient to induce long-lasting tolerance and avoid autoimmunity. Aire-controlled mechanisms of central tolerance are largely dispensable in the adult, as a previously tolerized T-cell pool can buffer newly generated autoreactive T cells that might emerge.

Autoimmune polyendocrine syndrome type I (APS-I) is a rare and severe monogenic disorder due to loss of function of the Aire gene, resulting in a failure of central (thymic) tolerance mechanisms and in multiorgan autoimmunity. The list of autoimmune targets is extremely vast, including endocrine (parathyroid, adrenals, islets, thyroid, ...) and non-endocrine (liver, bronchi, digestive tract, ...) systems. One of the unique features of the disease is the susceptibility to chronic mucocutaneous candidiasis, which is often the presenting sign in early childhood and highly prevalent in affected individuals, as compared to other involvements. This susceptibility to candidiasis is quite unique and is not associated with risk of infection with other pathogens. The general mechanism for the disease lies in the role of the Aire gene in directing the expression of self antigens in specialized cells of the thymic medulla (medullary thymic epithelial cells) where these self antigens allow for the elimination of highly self-reactive T lymphocytes [5]. Although this general mechanism has been demonstrated in human and animal systems, it could not easily provide a basis for the unique susceptibility to candidiasis. Here it is shown that antibodies to IL-17 cytokines, produced by Th17 CD4⁺ T cells [6], probably explains the candidiasis observed in APS-I patients. Similar data were presented in an accompanying paper [7], showing in addition that patients with thymomas, a situation of acquired defective central T-cell tolerance similarly have antibodies to Th17 cytokines. In addition to providing a mechanistic basis for candidiasis, these results give ground to clinical evaluation of immunosuppressive therapies such as ones targeting B cells in patients with APS-I [8].

Complementing this particular aspect of APS-I, the paper by Guerau-de-Arellano et al. addresses a fundamental question regarding the immune system of whether the neonatal period is unique as a

time when the immune system becomes tolerant to any (most?) antigens that it encounters. Increased autoimmunity with age could be viewed as an escape from these mechanisms, and failure to establish neonatal tolerance results in catastrophic autoimmune diseases such as APS-I or IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked). The discovery of Aire and the establishment of its role in central tolerance has greatly enhanced our understanding of immune tolerance. It turns out that the thymus continues to play a role throughout life, as exemplified by premature immune aging in young adults thymectomized during early childhood and autoimmune diseases arising in patients with thymomas [9]. In this study, the Mathis group used a clever system of inducible Aire transgene in a background of Aire knockout mice, allowing them to control Aire expression throughout development. Expression of Aire during fetal life only had a minimal effect in preventing autoimmunity, whereas expression of Aire during fetal and the first 3 weeks of life almost completely prevented autoimmunity. In addition, infusions of physiologically lymphopenic Aire-deficient neonates with adult T cells partially prevented the autoimmune pathology, whereas transient depletion of lymphocytes in protected adult mice worsened the pathology. Altogether, these results provide important and new understanding of the regulation of autoimmunity in the neonatal period. Study of neonatal T tolerance in subjects at risk for autoimmune diseases might result in new paradigms for the treatment or prevention of autoimmunity by targeting this specific period of life. If we ever find a way to cure APS-I, we will have to diagnose it intrauterinely or in early infancy.

Concepts revised

Bugs like sweets – with a little help from GRP78

The endothelial cell receptor GRP78 is required for mucormycosis pathogenesis in diabetic mice

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Background: Mucormycosis is a fungal infection of the sinuses, brain, or lungs that causes a mortality rate of at least 50% despite first-line therapy. Angioinvasion is a hallmark of mucormycosis infections and endothelial cell receptor(s) for fungi of the order Mucorales (the fungi that cause mucormycosis) are not determined. Patients with elevated available serum iron, including those with diabetic ketoacidosis (DKA), are uniquely susceptible to mucormycosis, suggesting a role of iron and glucose in regulating the expression of such a receptor.

Methods and Results: Glucose-regulated protein 78 (GRP78) was identified as a novel host receptor that mediates invasion and damage of human endothelial cells by *Rhizopus oryzae*, the most common etiologic species of Mucorales, but not *Candida albicans* or *Aspergillus fumigatus*. Elevated concentrations of glucose and iron, consistent with those seen during DKA, enhanced GRP78 expression and the resulting *R. oryzae* invasion and damage of endothelial cells in a receptor-dependent manner. Mice with DKA, which have enhanced susceptibility to mucormycosis, exhibited increased expression of GRP78 in sinus, lungs, and brain compared with normal mice. Finally, GRP78-specific immune serum protected mice with DKA from mucormycosis.

Conclusion: The results suggest a unique susceptibility of patients with DKA to mucormycosis and provide a foundation for the development of new therapeutic interventions for these deadly infections.

Infections, in particular fungal, are a hallmark of diabetes and an important cause of morbidity and mortality in diabetic patients. The most generally invoked mechanism for this increased susceptibility is the increased availability of glucose as a metabolic substrate for pathogen growth. This paper revisits this concept and shows that such simplistic views should be revised. The authors have analyzed the pathogenesis of *Rhizopus oryzae*, the pathogen involved in mucormycosis, a deadly fungal infection that can occur in immunocompromised patients and in diabetic ketoacidosis. They show that a normal endothelial protein with unknown physiological functions serves as receptor for the

fungus. Moreover, high glucose, low pH and high iron concentrations, all observed during diabetic ketoacidosis, concur to increase the expression of the receptor. GRP78 is a member of the HSP70 protein family and functions as a chaperone. It has also been reported as a receptor for a variety of ligands, including an angiogenesis inhibitor, an activated proteinase inhibitor, a synthetic peptide a dengue virus and a Coxsackievirus. These data demonstrate the plasticity of pathogens which have adapted to mammalian proteins and use them as unwanted receptors or adhesion molecules. We should refrain from simplistic explanations for phenomena we believe to understand. Last and more importantly, blocking GRP78 with an antibody prevents mucormycosis and might become a useful approach to treat this deadly condition.

Food for thought

Tell me who you cite, I will tell you who you are

How citation distortions create unfounded authority: analysis of a citation network

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Background: During the preparation and writing of manuscripts, authors must consider carefully the selection, completeness, and appropriateness of the articles referenced to ensure adequate and accurate citation as a necessity of scientifically and methodologically sound research. The aim of the paper was to understand belief in a specific scientific claim by studying the pattern of citations among papers stating it.

Methods: A complete citation network was constructed from all PubMed indexed English literature papers addressing the belief that β -amyloid, a protein accumulated in the brain in Alzheimer's disease, is produced by and injures skeletal muscle of patients with inclusion body myositis. Social network theory and graph theory were used to analyze this network. Citation bias, amplification, and invention, and their effects on determining authority were studied.

Results: The network contained 242 papers and 675 citations addressing the belief, with 220,553 citation paths supporting it. Unfounded authority was established by citation bias against papers that refuted or weakened the belief; amplification, the marked expansion of the belief system by papers presenting no data addressing it; and forms of invention such as the conversion of hypothesis into fact through citation alone. Extension of this network into text within grants funded by the National Institutes of Health and obtained through the Freedom of Information Act showed the same phenomena present and sometimes used to justify requests for funding.

Conclusion: The author concludes that citation is an impartial scholarly method and a powerful form of social communication. Distortions in its use including bias, amplification, and invention, can be used to generate information cascades resulting in unfounded authority of claims. Construction and analysis of a claim-specific citation network may clarify the nature of a published belief system and expose distorted methods of social citation.

Citations of peer-reviewed papers constitute one of the bases of medical writing and allow authors to make statements based on 'current knowledge' or on previously established facts in the literature. Since most journals allow only a limited number of references, authors invariably have to make choices in the references they use. Although we have all noticed distortions in citations in specific manuscripts, this is the first systematic approach to such a phenomenon. The author used a very limited research field (the role of β -amyloid in inclusion body myositis) and applied social networks techniques to address the question of how citation distortion can create what the author calls 'unfounded authority'. 242 papers generated a network of more than 220,000 citation paths. Ten authoritative papers were all from the same group with only 4 of them containing primary data. Six

papers with primary data that were critical of the hypothesis were seldom or never cited. Citation distortions were noted, as well as amplification over time and impact on NIH funding. Whether the same is true to other fields of science is not known and more studies like this will be needed in other research areas to establish how widespread the process is. In the field of clinical medicine, the recognition of such citation biases has led to the development of meta-analyses and systematic reviews where all primary evidence is re-evaluated and weighed appropriately. However, this has not been the case for basic science, or for mechanistic or diagnostic studies. The accompanying Editorial calls for systematic reviews included or available for all grant applications. In addition, calling the attention of authors on the fairness of the selection of papers they cite could be one step forward [10]. In the meantime, we should all pay attention to citation distortion as readers, reviewers or authors of manuscripts to avoid carrying over inaccurate information from one paper to the next as elegantly demonstrated here.

**Important for clinical practice
Beware of how you say it**

Effect of alternative summary statistics for communicating risk reduction on decisions about taking statins: a randomized trial

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Background: Different ways of presenting treatment effects can affect healthcare decisions but little is known about which presentations best help people make decisions consistent with their own values.

Methods: Six summary statistics for communicating coronary heart disease (CHD) risk reduction with statins were compared: relative risk reduction and five absolute summary measures – absolute risk reduction, number needed to treat, event rates, tablets needed to take, and natural frequencies. A randomized trial was conducted to determine which presentation would result in choices most consistent with participants' values. Adult volunteers were recruited who participated through an interactive website. Participants rated the relative importance of outcomes using visual analog scales (VAS). They were randomized to one of the six summary statistics and asked to choose whether to take statins based on this information. A relative importance score was calculated by subtracting the VAS for the downsides of taking statins from the VAS score for coronary heart disease. Logistic regression was used to determine the association between participants' relative importance score and their choice. 2,978 participants completed the study.

Results: Relative risk reduction resulted in a 21% higher probability of choosing to take statins over all values of relative importance score compared to the absolute summary statistics. This corresponds to a number needed to treat of 5, i.e. for every 5 participants shown the relative risk reduction, 1 additional participant chose to take statins, compared to the other summary statistics. There were no significant differences among the absolute summary statistics in the association between relative importance score and participants' decisions whether to take statins. Natural frequencies were best understood (86% reported they understood them well or very well), and participants were most satisfied with this information.

Conclusion: Presenting the benefits of taking statins as a relative risk reduction increases the likelihood of people accepting treatment compared to presenting absolute summary statistics, independent of the relative importance they attach to the consequences. Natural frequencies may be the most suitable summary statistic for presenting treatment effects, based on self-reported preference, understanding of and satisfaction with the information, and confidence in the decision.

Communication in modern medical practice is essential since patients are more and more associated with difficult choices offered by several possibilities of contemporary medicine. These choices are increasingly complex for physicians in their own field, and approaches to present them both to

healthcare professionals and to patients have been developed. However, communication is never neutral and we know from daily practice that the ways we explain treatment affect patients' choice. This study addressed the issue in a systematic way by randomizing volunteers to 6 groups who were presented with the same therapeutic scenario (10-year coronary heart disease of risk of 6% without statins and 30% relative reduction in the risk with statins) using 6 different methods: relative risk reduction, number needed to treat, tablets needed to treat, absolute risk reduction, event rate and natural frequencies. Individuals in the group that was presented with relative risk reduction selected the treatment in 74% of cases, as compared with ≈50% in all other groups. A clear limitation of the study is that the recruitment was internet-based, and the volunteering subjects were not really involved in the decision process. However, the majority of volunteers was above 40 years of age and therefore potentially implicated in similar choices. Although subjectivity is an important part of medical decision-making, healthcare professionals increasingly share evidence-based medical information with their patients to allow them to participate in medical decisions. This study shows that this is not enough and that we should pay attention not only to the information we provide but also how we provide it.

New concerns EPO and mortality in cancer patients

Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomized trials

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Background: Erythropoiesis-stimulating agents reduce anemia in patients with cancer and could improve their quality of life, but these drugs might increase mortality.

Methods: Meta-analysis of randomized controlled trials in which these drugs plus red blood cell transfusions were compared with transfusion alone for prophylaxis or treatment of anemia in patients with cancer. Data for patients treated with epoetin alfa, epoetin beta, or darbepoetin alfa were obtained and analyzed by independent statisticians using fixed-effects and random-effects meta-analysis. Analyses were by intention to treat. Primary endpoints were mortality during the active study period and overall survival during the longest available follow-up, irrespective of anticancer treatment, and in patients given chemotherapy. Tests for interactions were used to identify differences in effects of erythropoiesis-stimulating agents on mortality across pre-specified subgroups.

Results: Data from a total of 13,933 patients with cancer in 53 trials were analyzed. 1,530 patients died during the active study period and 4,993 overall. Erythropoiesis-stimulating agents increased mortality during the active study period (combined hazard ratio 1.17, 95% CI 1.06–1.30) and worsened overall survival (1.06, 1.00–1.12), with little heterogeneity between trials. 10,441 patients on chemotherapy were enrolled in 38 trials. The combined hazard ratio for mortality during the active study period was 1.10 (0.98–1.24), and 1.04 (0.97–1.11) for overall survival. There was little evidence for a difference between trials of patients given different anticancer treatments.

Conclusion: Treatment with erythropoiesis-stimulating agents in patients with cancer increased mortality during active study periods and worsened overall survival. The increased risk of death associated with treatment with these drugs should be balanced against their benefits.

The value of this paper is really to illustrate the power of high-quality meta-analyses to elucidate rare or difficult-to-detect events in a complex situation such as the treatment of cancer. Patients with cancer are often anemic and it has been debated whether erythropoiesis-stimulating agents that increase the comfort and quality of life of patients might increase mortality. 53 out of 102 eligible studies were selected and the meta-analysis on more than 14,000 patients showed a 17% increased

risk of death during treatment and 6% overall. For pediatric endocrinologists, the value of the study is not really to discuss whether the use of EPO is warranted during cancer treatment or not, but rather to show how large dataset analyses can increase our knowledge and provide answers to pending questions. The practical implications of such answers are then, as we just learned in the previous article, a matter of how the results are communicated.

Interesting mechanism

End of the chromosome and the end of life

Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians

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Context: Telomere length in humans is emerging as a biomarker of aging because its shortening is associated with aging-related diseases and early mortality. However, genetic mechanisms responsible for these associations are not known.

Methods: Here, in a cohort of Ashkenazi Jewish centenarians, their offspring, and offspring-matched controls, the inheritance and maintenance of telomere length and variations in two major genes associated with telomerase enzyme activity, hTERT and hTERC, were studied.

Results: The study demonstrated that centenarians and their offspring maintain longer telomeres compared with controls with advancing age and that longer telomeres are associated with protection from age-related diseases, better cognitive function, and lipid profiles of healthy aging. Sequence analysis of hTERT and hTERC showed overrepresentation of synonymous and intronic mutations among centenarians relative to controls. Moreover, they identified a common hTERT haplotype that is associated with both exceptional longevity and longer telomere length.

Conclusions: Thus, variations in human telomerase gene that are associated with better maintenance of telomere length may confer healthy aging and exceptional longevity in humans.

Telomeres consist of the TTAGGG tandem repeats at the ends of chromosomes and are known to protect these regions from degradation and DNA repair activities. Telomeres progressively shorten with each cell division in cultured primary human cells until a critically shortened length is achieved, upon which the cells enter replicative senescence. This study assessed the telomere length in blood leukocytes among subjects with exceptional longevity (centenarians) to investigate if centenarians survived with long telomeres, an indicator of better telomere length maintenance, or short telomeres reflecting chronologically old age. They demonstrated that not only centenarians, but their offspring too, maintain longer telomeres compared with controls with advancing age and that longer telomeres are associated with protection from age-related diseases, better cognitive function, and lipid profiles of healthy aging. They identified a common telomerase gene haplotype that is associated with both exceptional longevity and longer telomere length.

***Ardipithecus ramidus* and the paleobiology of early hominids**

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Context: Hominid fossils predating the emergence of *Australopithecus* have been sparse and fragmentary. The evolution of our lineage after the last common ancestor we shared with chimpanzees has therefore remained unclear.

Methods: *Ardipithecus ramidus*, recovered in ecologically and temporally resolved contexts in Ethiopia's Afar Rift, now illuminates earlier hominid paleobiology and aspects of extant African ape evolution. More than 110 specimens recovered from 4.4-million-year-old sediments include a partial skeleton with much of the skull, hands, feet, limbs, and pelvis.

Results: This hominid combined arboreal palmigrade clambering and careful climbing with a form of terrestrial bipedality more primitive than that of *Australopithecus*. *Ar. ramidus* had a reduced canine/premolar complex and a little-derived cranial morphology and consumed a predominantly C3 plant-based diet (plants using the C3 photosynthetic pathway). Its ecological habitat appears to have been largely woodland-focused. *Ar. ramidus* lacks any characters typical of suspension, vertical climbing, or knuckle-walking. *Ar. ramidus* indicates that despite the genetic similarities of living humans and chimpanzees, the ancestor we last shared probably differed substantially from any extant African ape.

Conclusions: Hominids and extant African apes have each become highly specialized through very different evolutionary pathways. This evidence also illuminates the origins of orthograde, bipedality, ecology, diet, and social behavior in earliest Hominidae and helps to define the basal hominid adaptation, thereby accentuating the derived nature of *Australopithecus*.

A special issue of *Science* was devoted to describe the eldest hominid, *Ardipithecus ramidus*, who roamed what is now Ethiopia 4.4 million years ago. The most complete skeleton of a female was nicknamed 'Ardi', who lived more than a million years before 'Lucy'. Ardi, who weighed about 50 kg and stood about 120 cm tall, had a mix of 'primitive' traits, shared with her predecessors, the primates of the Miocene epoch, and 'derived' traits, which it shares exclusively with later hominids. The investigators described her as a mosaic creature, i.e. neither chimpanzee nor human. Ardi lived in a woodland environment where she climbed on all fours along tree branches but walked, upright, on two legs, while on the ground.

We keep evolving

The following review and the subsequent four articles deal with ongoing evolution of us - modern humans

Has human evolution stopped?

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Context: It has been argued that human evolution has stopped because humans now adapt to their environment via cultural evolution and not biological evolution. However, all organisms adapt to their

environment, and humans are no exception. Culture defines much of the human environment, so cultural evolution has actually led to adaptive evolution in humans.

Evidence: Examples are given to illustrate the rapid pace of adaptive evolution in response to cultural innovations. These adaptive responses have important implications for infectious diseases, mendelian genetic diseases, and systemic diseases in current human populations. Moreover, evolution proceeds by mechanisms other than natural selection. The recent growth in human population size has greatly increased the reservoir of mutational variants in the human gene pool, thereby enhancing the potential for human evolution. The increase in human population size coupled with our increased capacity to move across the globe has induced a rapid and ongoing evolutionary shift in how genetic variation is distributed within and among local human populations. In particular, genetic differences between human populations are rapidly diminishing and individual heterozygosity is increasing, with beneficial health effects. Finally, even when cultural evolution eliminates selection on a trait, the trait can still evolve due to natural selection on other traits.

Conclusions: Our traits are not isolated, independent units, but rather are integrated into a functional whole, so selection on one trait can cause evolution to occur on another trait, sometimes with mildly maladaptive consequences.

The distinguished paleontologist Stephen Jay Gould stated: 'There's been no biological change in humans in 40,000 or 50,000 years. Everything we call culture and civilization we've built with the same body and brain.' Templeton argues that human evolution has not stopped, and our ongoing evolution has many medical and health implications. The rationale for the cessation of human evolution is based on the premise that cultural evolution eliminates adaptive evolution via natural selection. However, all organisms adapt to their environment, and in humans much of our environment is defined by our culture. Hence, cultural change can actually spur on adaptive evolution in humans. The only way to truly stop any biological organism from evolving is extinction. Evolution would be slowed by reducing and keeping population size to a small number of individuals. This will lead to a loss of most genetic variations through genetic drift and minimize the input of new mutations into the population. Our population size has been increasing over the last 10,000 years, and is now so large that the current human gene pool contains an immense reservoir of genetic variation; our evolutionary potential has never been higher. Our evolution is further driven by a radical change in the balance of genetic drift and gene flow that is rapidly causing a major evolutionary change in the human species in how genetic variation is distributed within and among local populations. Even when our cultural innovations do eliminate selection on a trait, that trait can still evolve as a correlated response to evolution of another trait, often in a non-adaptive fashion and sometimes in a mildly maladaptive fashion. As long as humans persist as a reproducing population, humans will evolve. This has been the lesson of the past 10,000 years, and is certainly what we can expect to continue for as long as our species persists on the Earth.

Natural selection in a contemporary human population

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Context: The aims of this study were to demonstrate that natural selection is operating on contemporary humans, predict future evolutionary change for specific traits with medical significance, and show that for some traits we can make short-term predictions about our future evolution.

Methods: To do so, they measured the strength of selection, estimated genetic variation and covariation, and predicted the response to selection for women in the Framingham Heart Study, a project of the National Heart, Lung, and Blood Institute and Boston University that began in 1948.

Results: They found that natural selection is acting to cause slow, gradual evolutionary change. The descendants of these women are predicted to be on average slightly shorter and stouter, to have lower total cholesterol levels and systolic blood pressure, to have their first child earlier, and to reach menopause later than they would in the absence of evolution.

Conclusions: Selection is tending to lengthen the reproductive period at both ends. To better understand and predict such changes, the design of planned large, long-term, multicohort studies should include input from evolutionary biologists.

Natural selection is acting slowly and gradually on traits of medical importance and on life history traits. The study examined the vital statistics of 2,238 postmenopausal women participating in the Framingham Heart Study, searching for correlations between women's height, weight, blood pressure and cholesterol levels and the number of offspring they produced. Both age at first birth and age at menopause appear to be changing so as to lengthen the reproductive period, which is consistent with previous findings. Slightly overweight (but not obese) women tended to have more children, as did women with lower blood pressure and cholesterol levels. Using a sophisticated statistical analysis that controlled for any social or cultural factors that could impact childbearing, the researchers determined that these characteristics were passed on genetically from mothers to daughters and granddaughters. It was suggested that if these trends were to continue with no cultural changes for the next 10 generations, by 2409 the average Framingham woman would be 2 cm (0.8 in) shorter, 1 kg (2.2 lb) heavier, have a healthier heart, have her first child 5 months earlier and enter menopause 10 months later than a woman today.

High frequency of lactose intolerance in a prehistoric hunter-gatherer population in northern Europe

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Context: Genes and culture are believed to interact, but it has been difficult to find direct evidence for the process. One candidate example that has been put forward is lactase persistence in adulthood, i.e. the ability to continue digesting the milk sugar lactose after childhood, facilitating the consumption of raw milk. This genetic trait is believed to have evolved within a short time period and to be related with the emergence of sedentary agriculture.

Results: This study investigated the frequency of an allele (−13910*T) associated with lactase persistence in a Neolithic Scandinavian population. From the 14 individuals originally examined, 10 yielded reliable results. They find that the T allele frequency was very low (5%) in this Middle Neolithic hunter-gatherer population, and that the frequency is dramatically different from the extant Swedish population (74%).

Conclusions: They conclude that this difference in frequency could not have arisen by genetic drift and is either due to selection or, more likely, replacement of hunter-gatherer populations by sedentary agriculturalists.

The ability to drink milk as an adult occurs at a high frequency in present-day Caucasians, who have been practicing dairying and cattle rearing for 10,000 years, and less so in African and Far Eastern populations who started farming only some 2,000 years ago. This capacity is closely associated with the transition from hunter-gatherer to agricultural societies. Apparently, the capacity to consume unprocessed milk into adulthood is regarded as having been of great significance for human prehistory. This has been regarded as an adaptive genetic trait exposed to positive selection induced by cultural practices. But the hunter-gatherers who inhabited Scandinavia 4,000 years ago were lactose-intolerant. They differed significantly from modern Swedes in terms of the DNA sequence that we generally associate with a capacity to digest lactose into adulthood. The paper suggests two possible explanations for the DNA differences. One possibility is that these differences are evidence of a powerful selection process, through which the Stone Age hunter-gatherers' genes were lost due to some significant advantage associated with the capacity to digest milk. The other is that today's Scandinavians are not descended from the Stone Age people in question, but from a group that arrived later. Most importantly, these findings describe a possible scenario where cultural practices could have had a tremendous impact on the genetic composition of human populations.

The origins of lactase persistence in Europe

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Context: Lactase persistence (LP) is common among people of European ancestry, but with the exception of some African, Middle Eastern and Southern Asian groups, is rare or absent elsewhere in the world. Lactase gene haplotype conservation around a polymorphism strongly associated with LP in Europeans (–13,910 C/T) indicates that the derived allele is recent in origin and has been subject to strong positive selection. Furthermore, ancient DNA work has shown that the –13,910*T (derived) allele was very rare or absent in early Neolithic Central Europeans. It is unlikely that LP would provide a selective advantage without a supply of fresh milk, and this has led to a gene-culture co-evolutionary model where LP is only favored in cultures practicing dairying, and dairying is more favored in lactase-persistent populations.

Methods: The study developed a flexible demic computer simulation model to explore the spread of LP, dairying, other subsistence practices and unlinked genetic markers in Europe and Western Asia's geographic space.

Results: Using data on –13,910*T allele frequency and farming arrival dates across Europe, and approximate bayesian computation to estimate parameters of interest, they infer that the –13,910*T allele first underwent selection among dairying farmers around 7,500 years ago in a region between the Central Balkans and Central Europe, possibly in association with the dissemination of the Neolithic Linearbandkeramik culture over Central Europe. Furthermore, the results suggest that natural selection favoring a LP allele was not higher in northern latitudes through an increased requirement for dietary vitamin D.

Conclusions: The results provide a coherent and spatially explicit picture of the co-evolution of LP and dairying in Europe.

Before the evolution of lactase persistence, humans typically lost their ability to digest lactose around the age of 5. This is thought to have helped motivate weaning. Still today, most of the world's population, including Asians and Africans, can only tolerate milk for the first few years of life. But, through at least four parallel evolutions starting several thousand years ago, lactase persistence spread throughout human populations. In Europe, a single genetic variant, –13,910*T, is strongly associated with lactase persistence and appears to have been favored by natural selection. Since adult consumption of fresh milk was only possible after the domestication of animals, it is likely that lactase persistence co-evolved with the cultural practice of dairying, although it is not known when lactase persistence first arose in Europe or what factors drove its rapid spread. Using a simulation model of the spread of lactase persistence, dairying, and farmers in Europe, integrating genetic and archaeological data the report shows that lactase persistence/dairying co-evolution began around 7,500 years ago in the Balkans and Central Europe, and not in the northern part of the continent, as previously thought.

Variants in neuropeptide Y receptors 1 and 5 are associated with nutrient-specific food intake and are under recent selection in Europeans

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Context: There is a large variation in caloric intake and macronutrient preference between individuals and between ethnic groups, and these food intake patterns show a strong heritability. The transition to new food sources during the agriculture revolution around 11,000 years ago probably created selective pressure and shaped the genome of modern humans. One major player in energy homeostasis is the appetite-stimulating hormone neuropeptide Y, in which the stimulatory capacity may be mediated by the neuropeptide Y receptors 1, 2 and 5 (NPY1R, NPY2R and NPY5R).

Methods: The study assesses association between variants in the NPY1R, NPY2R and NPY5R genes, and nutrient intake in a cross-sectional, single-center study of 400 men aged 40–80 years, and examines whether genomic regions containing these genes show signatures of recent selection in 270 HapMap individuals (90 Africans, 90 Asians, and 90 Caucasians) and in 846 Dutch blood bank controls.

Results: The results show that derived alleles in NPY1R and NPY5R are associated with lower carbohydrate intake, mainly because of a lower consumption of mono- and disaccharides. They also show that carriers of these derived alleles, on average, consume meals with a lower glycemic index (GI) and glycemic load (GL), and have higher alcohol consumption. One of these variants shows the hallmark of recent selection in Europe.

Conclusions: The data suggest that lower carbohydrate intake, consuming meals with a low GI and GL, and/or higher alcohol consumption, gave a survival advantage in Europeans since the agricultural revolution. This advantage could lie in overall health benefits, because lower carbohydrate intake, consuming meals with a low GI and GL, and/or higher alcohol consumption, are known to be associated with a lower risk of chronic diseases.

The appetite-stimulating hormone neuropeptide Y (NPY) evokes through the NPY receptors eating behavior, inducing particularly carbohydrate intake. Injection of NPY in the brain elicits a strong feeding response even in satiated animals, eventually leading to obesity. The results of this study show that derived alleles in NPY1R and NPY5R are associated with lower relative carbohydrate intake, mainly because of a lower consumption of mono- and disaccharides. Carriers of these derived alleles on average consume meals with a lower glycemic index (GI) and glycemic load (GL). However, the same alleles are associated with increased alcohol consumption. The derived allele appears to be under recent selection in the European population, and probably originates from around 4,000 years ago. A predicted selective sweep of around 4,000 years ago fits the theory of adaptation to novel food sources during the agriculture revolution, which started in Europe around 6,000 years ago and was gradually further developed from that point on. The data suggest that a lower carbohydrate intake, consumption of meals with a low GI and GL, and/or higher alcohol consumption gave a survival advantage in Europeans during the agricultural revolution.

***Homo floresiensis*: a cladistic analysis**

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Context: The announcement of a new species, *Homo floresiensis*, a primitive hominin that survived until relatively recent times, is an enormous challenge to paradigms of human evolution. Until this announcement, the dominant paradigm stipulated that: (1) only more derived hominins had emerged from Africa, and (2) *Homo sapiens* was the only hominin since the demise of *Homo erectus* and *Homo neanderthalensis*. Resistance to *H. floresiensis* has been intense, and debate centers on two sets of competing hypotheses: (1) that it is a primitive hominin, and (2) that it is a modern human, either a pygmoid form or a pathological individual. Despite a range of analytical techniques having been applied to the question, no resolution has been reached.

Methods: Here, they use cladistic analysis, a tool that has not, until now, been applied to the problem, to establish the phylogenetic position of the species.

Results: The results produce two equally parsimonious phylogenetic trees. The first suggests that *H. floresiensis* is an early hominin that emerged after *Homo rudolfensis* (1.86 Ma) but before *Homo habilis* (1.66 Ma, or after 1.9 Ma if the earlier chronology for *H. habilis* is retained). The second tree indicates *H. floresiensis* branched after *H. habilis*.

In 2005, the *Yearbook* enthusiastically discussed the finding of a 20,000-year ancient hominin in Indonesia with a stature of 1 m and a small skull. Following several years of a debate whether this was a diseased *H. sapiens* or a new species, this series of articles conclude that it was indeed a new species. We were naive to assume that we all come from Africa during the 100–40,000 years ago ‘out of Africa’ migration. Body size reduction in mammals is usually associated with only moderate brain size reduction, because the brain and sensory organs complete their growth before the rest of the

body during ontogeny. But the small skull of *H. floresiensis* may teach us otherwise. Indeed, this trend has been questioned in the special case of dwarfism of mammals on islands, the so-called insular dwarfism. The nature of the proportional change in brain size in insular dwarfism indicates that selective pressures on brain size are potentially independent of those on body size.

Concepts revised If injured, run in bare feet

Foot strike patterns and collision forces in habitually barefoot versus shod runners

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Background: Humans have engaged in endurance running for millions of years, but the modern running shoe was not invented until the 1970s. For most of human evolutionary history, runners were either barefoot or wore minimal footwear such as sandals or moccasins with smaller heels and little cushioning relative to modern running shoes. The authors wondered how runners coped with the impact caused by the foot colliding with the ground before the invention of the modern shoe.

Methods: Kinematic studies of foot strike in 5 groups of runners habitually shod or unshod.

Results: Habitually barefoot endurance runners often land on the fore-foot (fore-foot strike) before bringing down the heel, but they sometimes land with a flat foot (mid-foot strike) or, less often, on the heel (rear-foot strike). In contrast, habitually shod runners mostly rear-foot strike, facilitated by the elevated and cushioned heel of the modern running shoe. Kinematic and kinetic analyses show that even on hard surfaces, barefoot runners who fore-foot strike generate smaller collision forces than shod rear-foot strikers. This difference results primarily from a more plantar-flexed foot at landing and more ankle compliance during impact, decreasing the effective mass of the body that collides with the ground.

Conclusion: Fore-foot- and mid-foot-strike gaits were probably more common when humans ran barefoot or in minimal shoes, and may protect the feet and lower limbs from some of the impact-related injuries now experienced by a high percentage of runners.

We generally tend to think that modern life utensils protect us from injuries and provide us with improved performances. This study, reminiscent of the Noble Savage theory [11], reminds us that human body evolution over millenaries cannot be 'improved' in 40 years by designers from Nike®, Reebok® and consorts. The authors are evolutionary biologists, bioengineers and physical medicine specialists and observed foot impact of various groups of runners while shod or unshod. 'Modern' usually shod runners mostly strike the heel first, while unshod runners from Kenya mostly strike the fore-foot first. The shod heel first pattern resulted in more repetitive high impact forces while unshod running was associated with a more diverse way of running, resulting in a lessened risk of injury. The findings of the study complement the endurance-running hypothesis for the transformation of the human body plan with the emergence of the genus *Homo*. The much earlier australopithecine version of bipedalism (as seen in 'Lucy', *Australopithecus afarensis*) received a major makeover near the Pliocene/Pleistocene boundary about 2 million years ago with longer hind limbs and shorter toes. If the endurance-running hypothesis is correct, the evolution of these features are probably linked directly to barefoot running as an integral part of an adaptive strategy for pursuit hunting [12]. Many shod modern-day runners develop injuries [13] and new footwear now use barefoot like design and might protect them from repetitive stress injury. Nowadays, every big marathon like New York, Paris or Berlin brings together 30,000–40,000 participants somehow holding an atavistic ceremony commemorating their ancient past. We might see them leaving their shoes in the locker room before long.

MiR-33 contributes to the regulation of cholesterol homeostasis

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Background: Cholesterol metabolism is tightly regulated at the cellular level.

Results: miR-33, an intronic microRNA (miRNA) located within the gene encoding sterol-regulatory element-binding factor-2 (SREBF-2), a transcriptional regulator of cholesterol synthesis, modulates the expression of genes involved in cellular cholesterol transport. In mouse and human cells, miR-33 inhibits the expression of the adenosine triphosphate-binding cassette (ABC) transporter, ABCA1, thereby attenuating cholesterol efflux to apolipoprotein A1. In mouse macrophages, miR-33 also targets ABCG1, reducing cholesterol efflux to nascent high-density lipoprotein (HDL). Lentiviral delivery of miR-33 to mice represses ABCA1 expression in the liver, reducing circulating HDL levels. Conversely, silencing of miR-33 in vivo increases hepatic expression of ABCA1 and plasma HDL levels.

Conclusion: miR-33 regulates both HDL biogenesis in the liver and cellular cholesterol efflux.

MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis

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Background: Proper coordination of cholesterol biosynthesis and trafficking is essential to human health. The sterol regulatory element-binding proteins (SREBPs) are key transcription regulators of genes involved in cholesterol biosynthesis and uptake.

Results: MicroRNAs (miR-33a/b) embedded within introns of the SREBP genes target the adenosine triphosphate-binding cassette transporter A1 (ABCA1), an important regulator of high-density lipoprotein (HDL) synthesis and reverse cholesterol transport, for posttranscriptional repression. Antisense inhibition of miR-33 in mouse and human cell lines causes up-regulation of ABCA1 expression and increased cholesterol efflux, and injection of mice on a Western-type diet with locked nucleic acid-antisense oligonucleotides results in elevated plasma HDL.

Conclusion: miR-33 acts in concert with the SREBP host genes to control cholesterol homeostasis. miR-33 may represent a therapeutic target for ameliorating cardiometabolic diseases.

PubMed provides 8,186 citations to microRNA, which were discovered as late as 2001. They have emerged as essential players in gene regulation. Initially identified in the cancer field, which still constitutes three quarters of microRNA literature, they are now recognized as essential players in gene expression in all tissues. MicroRNAs are small (22-nt) endogenous double-stranded RNAs that regulate physiological processes at the posttranscriptional level [14, 15]. They bind to complementary target sites in the 3' untranslated regions of mRNAs, causing translational repression and/or mRNA destabilization [3]. A single microRNA can have multiple targets, potentially regulating several genes involved in a physiological pathway. miR-122 has been previously implicated in cholesterol regulation [16]. These two papers further extend the role of microRNAs in metabolic regulation and miR-33 appears as a key regulator of HDL cholesterol metabolism. Using different approaches, the authors identified two isoforms of miR-33 that are embedded in introns of SREBP genes 1 & 2, encoding sterol regulatory element-binding proteins which in turn regulate the synthesis of fatty acids and cholesterol respectively. miR-33 targets ABCA1, a regulator of HDL synthesis, and implicated in Tangier disease, a condition of plasma HDL deficiency. The studies demonstrate an impact on macrophages and, to a lesser extent, on the liver. In vivo manipulation of miR-33 levels using lentiviral vectors increased plasma HDL levels by 25% using anti-miR-33 and decreased plasma HDL levels by 22% using miR-33. Decreased plasma HDL is a key feature of the metabolic syndrome, and miR-33 could

play a central role downstream of the enhanced liver insulin signaling of the metabolic syndrome. Quite interestingly, the authors show that individuals with mutations in miR-33b might be protected from decreased HDL and the metabolic syndrome. Agents that antagonize miR-33 will have to be tested to evaluate their potential to increase plasma HDL levels and treat the metabolic syndrome.

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