Science and Medicine

Ze'ev Hochberg and Ken Ong

Urgent need to understand our biosphere

Approaching a state shift in Earth's biosphere

Barnosky AD, Hadly EA, Bascompte J, Berlow EL, Brown JH, Fortelius M, Getz WM, Harte J, Hastings A, Marquet PA, Martinez ND, Mooers A, Roopnarine P, Vermeij G, Williams JW, Gillespie R, Kitzes J, Marshall C, Matzke N, Mindell DP, Revilla E, Smith AB

Department of Integrative Biology, University of California, Berkeley, CA, USA

barnosky@berkeley.edu

Nature 2012;486:52–58

Background: Localized ecological systems are known to shift abruptly and irreversibly from one state to another when they are forced across critical thresholds.

Methods and Results: The authors reviewed the evidence that the global ecosystem as a whole can react in the same way and is approaching a planetary-scale critical transition as a result of human influence. The plausibility of a planetary-scale 'tipping point' highlights the need to improve biological forecasting by detecting early warning signs of critical transitions on global as well as local scales, and by detecting feedbacks that promote such transitions.

Conclusions: It is necessary to address root causes of how humans are forcing biological changes.

An international group of scientists joined forces to deliver a warning that, unless steps are taken, population growth, destruction of natural ecosystems, and climate change will impact on much of what we depend on to sustain our quality of life, including, for example, fisheries, agriculture, forest products and clean water. This could happen within just a few generations. This paper describes an urgent need to understand how the biosphere reacted in the distant past to rapidly changing conditions, including climate and human population growth. The authors argue that, although many warning signs are emerging, no one knows how close the Earth is to a global tipping point, or whether it is inevitable. Currently, about 43% of the Earth's land surface has been converted to agricultural or urban use, with roads cutting through much of the remainder. They conclude that there is an urgent need for global cooperation to reduce world population growth and per-capita resource use, replace fossil fuels with sustainable sources, develop more efficient food production and distribution without taking over more land, and better manage the land and ocean areas not already dominated by humans, as reservoirs of biodiversity and ecosystem services.

Genetics of human adaptation

Evolutionary history and adaptation from high-coverage whole-genome sequences of diverse African hunter-gatherers

Lachance J, Vernot B, Elbers CC, Ferwerda B, Froment A, Bodo JM, Lema G, Fu W, Nyambo TB, Rebbeck TR, Zhang K, Akey JM, Tishkoff SA

Department of Biology, University of Pennsylvania, Philadelphia, PA, USA

Cell 2012;150:457–469

Background: The aim of the study was to reconstruct modern human evolutionary history and identify genetic loci that have shaped hunter-gatherer adaptation.

Methods: The authors performed >60-fold coverage whole-genome sequencing of 5 individuals in each of three different hunter-gatherer populations: Pygmies from Cameroon, and Khoesan-speaking Hadza and Sandawe from Tanzania.

Results and Conclusions: 13.4 million variants were identified, which represents a substantial increase in the set of known human variation. There was evidence of archaic introgression in all three populations, and the distribution of time to most recent common ancestors from these regions is similar to that observed for introgressed regions in Europeans. In addition, numerous loci were identified that harbor signatures of local adaptation, including genes involved in immunity, metabolism, olfactory and taste perception, reproduction, and wound healing. Within the Pygmy population, multiple highly differentiated loci were identified that play a role in anterior pituitary function and growth, and are associated with height.

The authors aimed to extend our knowledge of human genetic diversity and thereby also: (i) gain a broader understanding of functional genetic diversity and disease susceptibility, and (ii) inform the genetic basis of adaptation to diverse environments. Previous limited genetic data in these African populations showed they have an ancient divergence, even though two, the Hadza and Sandawe, are geographical close neighbors (~150 km apart). Now with whole-genome sequencing, the authors found multiple genomic regions that showed marked divergence between the groups, and these regions contained genes with a remarkable range of functions. Evolutionary geneticists interpret such divergence as genetic adaptations to the differences in local diets, pathogens, and environments of these populations, who have sustained their unique cultures of hunting and gathering. In addition, genes with high-frequency variants exclusive to the Pygmy population included *TRHR* (thyrotropin-releasing hormone receptor), *HESX1* and *POU1F1* (anterior pituitary development), *APPL1* (adiponectin and insulin signaling pathways), *FSHR* (follicle-stimulating hormone receptor) and genes associated with Williams syndrome. These findings suggest that regulation of pituitary function, height, reproduction and metabolism played a role in local adaptation in Pygmies. Finally, such studies also shed light on human evolutionary history. Introgression is the flow of genes from one species to another and back again. In all three populations, low levels of introgression from an archaic population were found, supporting the existence of a yet unknown and now extinct form of the genus *Homo* in Central Africa, who interbred with anatomically modern humans.

Our Denisovan cousin

A high-coverage genome sequence from an archaic Denisovan individual

Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, Mallick S, Schraiber JG, Jay F, Prufer K, de Filippo C, Sudmant PH, Alkan C, Fu Q, Do R, Rohland N, Tandon A, Siebauer M, Green RE, Bryc K, Briggs AW, Stenzel U, Dabney J, Shendure J, Kitzman J, Hammer MF, Shunkov MV, Derevianko AP, Patterson N, Andres AM, Eichler EE, Slatkin M, Reich D, Kelso J, Paabo S

Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany
mmeyer@eva.mpg.de

Science 2012;338:222–226

Background: This paper presents a DNA library preparation method that has allowed the authors to reconstruct a high-coverage (30×) genome sequence of a Denisovan, an extinct relative of Neandertals.

Methods: The quality of this genome allows a direct estimation of Denisovan heterozygosity, indicating that genetic diversity in these archaic hominins was extremely low.

Results and Conclusions: This genome allows tentative dating of the specimen on the basis of ‘missing evolution’ in its genome, detailed measurements of Denisovan and Neandertal admixture into present-day human populations, and the generation of a near-complete catalog of genetic changes that swept to high frequency in modern humans since their divergence from Denisovans.

Two years ago, the *Yearbook* described the publishing of the genome sequence of the Neanderthal, and last year that of the Aboriginal dwellers of Australia. This year we draw attention to the genome of the Denisovans, an archaic human who lived in Siberia at least 50,000 years ago. It all started in March 2010, when scientists announced the discovery of a finger bone fragment of a girl who lived in the Denisova Cave in the Altai Mountains in Siberia, a cave which has also been inhabited by

Neanderthals and modern humans. A tooth and toe bone belonging to different members of the same population has since been found. The amazing resolution of this technique allows us a glimpse of the girl, who died between 74,000 and 82,000 years ago. She had brown eyes, brown hair, and brown skin. This article is also remarkable for the new method that enabled the authors to provide a genome resolution comparable to that from the DNA of living people. It will surely give us glimpses of other archaic ancestors.

An abstract thought

U-series dating of Paleolithic art in 11 caves in Spain

Pike AW, Hoffmann DL, Garcia-Diez M, Pettitt PB, Alcolea J, De Balbin R, Gonzalez-Sainz C, de las Heras C, Lasheras JA, Montes R, Zilhao J
Department of Archaeology and Anthropology, University of Bristol, Bristol, UK
alistair.pike@bristol.ac.uk

Science 2012;336:1409–1413

Background: Paleolithic cave art is an exceptional archive of early human symbolic behavior. However, the difficulty in obtaining reliable dating means that its chronology remains poorly understood even after more than a century of study.

Methods: The authors performed uranium-series disequilibrium dating of calcite deposits overlying or underlying art found in 11 caves, including the United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage sites of Altamira, El Castillo, and Tito Bustillo, Spain.

Results: The results demonstrate that the tradition of decorating caves extends back at least to the Early Aurignacian period, with minimum ages of 40.8 thousand years for a red disk, 37.3 thousand years for a hand stencil, and 35.6 thousand years for a claviform-like symbol.

Conclusions: These minimum ages reveal either that cave art was a part of the cultural repertoire of the first anatomically modern humans in Europe, or that perhaps Neanderthals also engaged in painting caves.

The cave art of Altamira, El Castillo and Tito Bustillo is now estimated to be more than 40,000 years old. This date coincides with the first known immigration into Europe of *Homo sapiens*. If anatomically modern humans were responsible, then it means they engaged in the activity almost immediately on their arrival in Europe. The authors alternatively suggest that the Neanderthals were the artisans, which is even more intriguing. Works of art require a high degree of abstract thought and behavior. We used to believe that symbolism – the ability to let one thing represent another in the mind – is a unique human trait. This pretentious thinking leads us to the next paper.

Conscious thoughts

A prominent international group of cognitive neuroscientists, neuropharmacologists, neurophysiologists, neuroanatomists and computational neuroscientists gathered at the University of Cambridge to reassess the neurobiological substrates of conscious experience and related behaviors in human and non-human animals. It provides important insight into how we treat animals in the laboratories and the food industry. Their report appears in the following webpage, and is abbreviated hereby.

The Cambridge Declaration on Consciousness

<http://fcmconference.org/img/CambridgeDeclarationOnConsciousness.pdf>

Background and Methods: Comparative research on conscious experience is hampered by obvious difficulties in studying non-humans. However, in animal models homologous brain circuits correlated with conscious experience and perception can be selectively facilitated or disrupted to assess whether they are

necessary for those experiences. Moreover, in humans, new non-invasive techniques are readily available to survey the correlates of consciousness.

Results: From such studies, the following observations can be stated unequivocally:

– The neural substrates of emotions are not confined to cortical structures. Rather, subcortical neural networks aroused during affective states in humans are also critically important for generating emotional behaviors in animals.

– Wherever in the brain one evokes instinctual emotional behaviors in non-human animals, many of the ensuing behaviors are consistent with experienced feeling states, including those internal states that are rewarding and punishing.

– Birds appear to offer, in their behavior, neurophysiology and neuroanatomy, a striking case of parallel evolution of consciousness. Evidence of near human-like levels of consciousness has been most dramatically observed in African grey parrots. Mammalian and avian emotional networks and cognitive microcircuitries appear to be far more similar than previously thought. Certain bird species exhibit mammalian-like neural sleep patterns, including REM sleep. Zebra finches show neurophysiological patterns, previously thought to require a mammalian neocortex. Magpies exhibit striking similarities to humans, great apes, dolphins, and elephants in studies of mirror self-recognition.

Conclusions: The absence of a neocortex does preclude the experience of affective states. Convergent evidence indicates that non-human animals have the neuroanatomical, neurochemical, and neurophysiological substrates of conscious states along with the capacity to exhibit intentional behaviors. The weight of evidence indicates that humans are not unique in possessing the neurological substrates that generate consciousness. Non-human animals, including all mammals and birds, and many other creatures, including octopuses, also possess these neurological substrates.

A birth control pill for men

Small-molecule inhibition of BRDT for male contraception

Matzuk MM, McKeown MR, Filippakopoulos P, Li Q, Ma L, Agno JE, Lemieux ME, Picaud S, Yu RN, Qi J, Knapp S, Bradner JE

Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA

mmatzuk@bcm.edu

Cell 2012;150:673–684

Background: A pharmacologic approach to male contraception remains a longstanding medical challenge.

Methods: The authors explored the spermatogenic effects of a selective small-molecule inhibitor (JQ1) of the bromodomain and extraterminal (BET) subfamily of epigenetic reader proteins.

Results: JQ1 potently inhibits the testis-specific member BRDT, which is essential for chromatin remodeling during spermatogenesis. Biochemical and crystallographic studies confirmed that occupancy of the BRDT acetyl-lysine binding pocket by JQ1 prevents recognition of acetylated histone H4. Treatment of mice with JQ1 reduced seminiferous tubule area, testis size, and spermatozoa number and motility without affecting hormone levels. Although JQ1-treated males mate normally, inhibitory effects of JQ1, evident at the spermatocyte and round spermatid stages, cause a complete and reversible contraceptive effect.

Conclusions: These data establish a new contraceptive that can cross the blood:testis boundary and inhibit bromodomain activity during spermatogenesis, providing a lead compound targeting the male germ cell for contraception.

There has not been a new reversible contraceptive for men since the development of the condom. This is the first effective non-hormonal male birth control pill for mice. The small-molecule inhibitor JQ1 makes male mice reversibly infertile without any adverse effect on their sex drive. The authors envision that their discoveries can be completely translated to men.

Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study

Mattison JA, Roth GS, Beasley TM, Tilmont EM, Handy AM, Herbert RL, Longo DL, Allison DB, Young JE, Bryant M, Barnard D, Ward WF, Qi W, Ingram DK, de Cabo R

Laboratory of Experimental Gerontology, National Institute on Aging, NIH Animal Center, Dickerson, MD, USA

mattisonj@mail.nih.gov

Nature 2012;489:318–321

Background: Calorie restriction (CR), a 10–40% reduction in intake of a nutritious diet, is often reported as the most robust non-genetic mechanism to extend lifespan and healthspan. CR is frequently used to study the mechanisms behind ageing and age-associated diseases. Independent of lifespan, CR has also been reported to delay or prevent the occurrence of many chronic diseases in a variety of animals. In rhesus monkeys, beneficial effects of CR on immune function, motor coordination and resistance to sarcopenia have recently been reported.

Methods and Results: The authors found that a CR regimen implemented in young and older age rhesus monkeys at the National Institute on Aging (NIA) did not improve survival.

Discussion: The findings contrast with an ongoing study at the Wisconsin National Primate Research Center (WNPRC), which reported improved survival associated with 30% CR initiated in adult rhesus monkeys (7–14 years) and a preliminary report in a small number of CR monkeys. Over the years, both NIA and WNPRC have extensively documented beneficial health effects of CR in these two apparently parallel studies. The implications of the WNPRC findings were important as they extended CR findings beyond the laboratory rodent and to a long-lived primate. The NIA study suggests there is a separation between the effects of CR on health and survival. Similar to studies in rodents, in a long-lived nonhuman primate, study design, husbandry and diet composition may strongly affect the life-prolonging effect of CR.

There have been many studies across many species showing that caloric restriction increases lifespan versus ad lib feeding. Despite the absence of any long-term controlled studies in humans, some of our friends have opted to live a caloric restriction lifestyle. The current NIA study began 25 years ago, feeding rhesus monkeys only 70% of the calories in a normal maintenance diet. In contrast to other studies, old caloric-restricted monkeys lived no longer than the controls, and showed no decrease in the incidence of cancer, cardiovascular disease, amyloidosis or general organism deterioration. Even though survival was not affected, the caloric-restricted monkeys had some metabolic benefits and the young-onset CR animals were healthier. The authors believe that differences in the diet and animal husbandry techniques may account for the difference from prior trials; the NIA study gave both control and study animals portions that did not allow them to eat enough to get obese and the study diet had only 3.9% sucrose content.

Correcting Down syndrome trisomy

Trisomy correction in Down syndrome induced pluripotent stem cells

Li LB, Chang KH, Wang PR, Hirata RK, Papayannopoulou T, Russell DW

Department of Medicine, University of Washington, Seattle, WA, USA

Cell Stem Cell 2012;11:615–619

Background: Human trisomies can alter cellular phenotypes and produce congenital abnormalities such as Down syndrome (DS).

Methods: The authors generated induced pluripotent stem cells (iPSCs) from DS fibroblasts and introduced a TKNEO transgene into one copy of chromosome 21 by gene targeting.

Results: When selecting against TKNEO, spontaneous chromosome loss was the most common cause for survival, with a frequency of $\sim 10^{-4}$, while point mutations, epigenetic silencing, and TKNEO

deletions occurred at lower frequencies in this unbiased comparison of inactivating mutations. Mitotic recombination events resulting in extended loss of heterozygosity were not observed in DS iPSCs. The derived, disomic cells proliferated faster and produced more endothelia in vivo than their otherwise isogenic trisomic counterparts, but in vitro hematopoietic differentiation was not consistently altered.

Conclusions: This study describes the targeted removal of a human trisomy, which could prove useful in both clinical and research applications.

This promising study removed the extra copy of chromosome 21 in cell cultures belonging to a person with Down syndrome (DS). Potentially, this work may apply far beyond DS; extra chromosomes account for almost a quarter of all spontaneous miscarriages. The researchers got the idea while working with stem cells to develop regenerative therapies, which often results in trisomies. They inserted a TKNEO fusion gene, encoding both thymidine kinase and neomycin resistance, into the chromosome 21 gene APP, to enable cells to respond to positive and negative selection under specific conditions. Selecting against TKNEO expression, by culturing the cells in the presence of ganciclovir, the only cells that survived were those that spontaneously lost the extra chromosome.

Early life events have clear consequences

A critical period for social experience-dependent oligodendrocyte maturation and myelination

Makinodan M, Rosen KM, Ito S, Corfas G

F.M. Kirby Neurobiology Center, Children's Hospital, Boston, MA, USA

Science 2012;337:1357–1360

Background: Early social isolation results in adult behavioral and cognitive dysfunction that correlates with white matter alterations. However, how social deprivation influences myelination and the significance of these myelin defects in the adult remained undefined.

Methods and Results: Mice isolated for 2 weeks immediately after weaning have alterations in prefrontal cortex function and myelination that do not recover with reintroduction into a social environment. These alterations, which occur only during this critical period, are phenocopied by loss of oligodendrocyte ErbB3 receptors, and social isolation leads to reduced expression of the ErbB3 ligand neuregulin-1.

Conclusions: These findings indicate that social experience regulates prefrontal cortex myelination through neuregulin-1/ErbB3 signaling and that this is essential for normal cognitive function, thus providing a cellular and molecular context to understand the consequences of social isolation.

Early life events have clear consequences. Thus, juvenile social isolation and neglect influence adult cognitive function and social interactions. Studies of children raised in institutions where neglect was widespread showed that deprivation also correlates with alterations in white matter tracts associated with the medial prefrontal cortex, which are not reversed by subsequent foster care placement. This study looks into the mechanisms by which social experience affect oligodendrocytes for the establishment of normal adult neuronal circuits and their function, and which molecular mechanisms underlie these events. The findings indicate that the effects of childhood isolation and neglect on adult mental health might be caused, at least in part, by alterations in oligodendrocytes and myelin development. The results may be relevant to neuropsychiatric disorders such as schizophrenia and mood disorders, which usually manifest after the juvenile period and have been linked to alterations in white matter and myelination. In this respect, it is noteworthy that the NRG1-ErbB signaling pathway has been genetically linked to neuropsychiatric disorders, which suggests that genetic liabilities in this pathway and life experience may interact in the pathogenesis of these diseases.

Low fertility increases descendant socioeconomic position but reduces long-term fitness in a modern post-industrial society

Goodman A, Koupil I, Lawson DW

Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK

anna.goodman@lshtm.ac.uk

Proc Biol Sci 2012;279:4342–4351

Background: Adaptive accounts of modern low human fertility argue that small family size maximizes the inheritance of socioeconomic resources across generations and may consequently increase long-term fitness.

Methods: The authors explored the long-term impacts of fertility and socioeconomic position (SEP) on multiple dimensions of descendant success in a unique Swedish cohort of 14,000 individuals born during 1915–1929.

Results: Low fertility and high SEP predicted increased descendant socioeconomic success across four generations. Furthermore, these effects were multiplicative; the greatest benefits of low fertility were observed in high SEP families. Low fertility and high SEP do not, however, predict increased descendant reproductive success.

Conclusions: These results are consistent with the idea that modern fertility limitation represents a strategic response to the local costs of rearing socioeconomically competitive offspring, but contradict adaptive models suggesting that it maximizes long-term fitness. This indicates a conflict in modern societies between behaviors promoting socioeconomic versus biological success. This study also makes a methodological contribution, demonstrating that the number of offspring strongly predicts long-term fitness and thereby validate use of fertility data to estimate current selective pressures in modern populations. Finally, the findings highlight that differences in fertility and SEP can have important long-term effects on the persistence of social inequalities across generations.

This is well known: family size generally falls as societies become richer. With an evolutionary perspective, this observation is counterintuitive: natural selection is expected to favor organisms that try to maximize their reproduction. The ‘adaptive’ hypothesis proposed that low fertility may boost evolutionary success in the long term by increasing offspring wealth, which in turn eventually increases the number of long-term descendants because richer offspring end up having more children. This article rejects that contention.

In a Swedish cohort from the early 1900s, the authors find that having fewer children increased economic success and social position of descendants for up to four generations, but reduced the total number of long-term descendants. Among both male and female offspring of the original cohort, smaller family size and higher parental socioeconomic position were both associated with substantially higher school marks, university entrance and income. These effects were particularly large when low fertility and high socioeconomic status coincided, with the benefits of small family size being particularly marked in wealthier groups. Moreover, these advantages were in turn passed on to the grandchild and great-grandchild generations. The authors conclude that the decision to limit family size can be understood as a strategic choice to improve the socioeconomic success of children and grandchildren in modern societies, but this socioeconomic benefit does not necessarily translate into an evolutionary benefit.

There seems to be a conflict in modern societies between behaviors promoting social and economic benefits versus biological success. One of the most interesting findings is the interaction between family size and wealth. Poorer households have relatively little to gain by limiting fertility, perhaps because the success of their children is more determined by broader societal factors, rather than the limited investment and inheritance available from their parents. This observation suggests an economic rationality to fertility patterns in the modern world, a consideration unknown to our predecessors.

Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units

Saunders CJ, Miller NA, Soden SE, Dinwiddie DL, Noll A, Alnadi NA, Andraws N, Patterson ML, Krivohlavek LA, Fellis J, Humphray S, Saffrey P, Kingsbury Z, Weir JC, Betley J, Grocock RJ, Margulies EH, Farrow EG, Artman M, Safina NP, Petrikin JE, Hall KP, Kingsmore SF

Center for Pediatric Genomic Medicine, Department of Pediatrics, Children's Mercy Hospital, School of Medicine, University of Missouri-Kansas City, Kansas City, MO, USA

Sci Transl Med 2012;4:154ra135

Background: Monogenic diseases are common causes of neonatal morbidity and mortality, and their presentations are often ambiguous at birth. Clinical testing is available for only a fraction of the currently characterized 3,500 monogenic diseases, and many conditions feature clinical and genetic heterogeneity. Hence, there is an immense need for improved molecular diagnosis in infants. Because disease progression may be extremely rapid in newborns, molecular diagnoses must be quickly made to inform clinical decision-making.

Methods: The authors describe the differential diagnosis of genetic disorders by whole-genome sequencing (WGS) with a 50-hour turn-around time, using an automated bioinformatic analysis that is intended to be a prototype for use in neonatal intensive care units.

Results: Retrospective 50-hour WGS identified known molecular diagnoses in 2 children. Prospective WGS disclosed the potential molecular diagnosis of a severe GJB2-related skin disease in 1 neonate; BRAT1-related lethal neonatal rigidity and multifocal seizure syndrome in another infant; identified BCL9L as a novel, recessive visceral heterotaxy gene (HTX6) in a pedigree, and ruled out known candidate genes in 1 infant. Sequencing of parents or affected siblings expedited the identification of disease genes in prospective cases.

Conclusions: Rapid WGS can potentially broaden and expedite the differential diagnosis of congenital disease in newborns. Potential benefits include fewer empirical treatments and faster progression to genetic and prognostic counseling.

Next-generation sequencing technologies have already revolutionized our research approaches to discover new genes involved in monogenic diseases, cancer and even complex diseases, such as obesity and type 2 diabetes. The application of these technologies into routine clinical practice is surely only a short step away. Indeed, it is particularly welcome to see new powerful technology being applied first to meet the needs of pediatric medicine. The authors argue that neonatal intensive care units are especially suitable for the early adoption of whole-genome sequencing (WGS) because of the relatively high burden of monogenic diseases in their patients; in the United States, more than 20% of infant deaths are caused by congenital malformations and other genetic diseases. For conditions where treatment is available, rapid diagnosis can reduce morbidity and mortality. Even if treatment is not yet available, early diagnosis can spare endless investigations and expedite genetic counseling.

The technological breakthrough described in this paper is the near-full automation of processes from WGS data generation, due to faster sample processing, imaging and chemistry, and automated characterization of genome variation and bioinformatic analysis of the results, shortening the process from 4–6 weeks to within 50 h. A major strategy was to focus the automated data analysis only to the parts of the genome relevant to the individual patient's presentation. This approach also has important ethical benefits for the patient and wider family, as described in the timely clinical guidelines for genetic testing and screening of children (see Editor's Choice).

Non-invasive whole-genome sequencing of a human fetus

Kitzman JO, Snyder MW, Ventura M, Lewis AP, Qiu R, Simmons LE, Gammill HS, Rubens CE, Santillan DA, Murray JC, Tabor HK, Bamshad MJ, Eichler EE, Shendure J

Department of Genome Sciences, University of Washington, Seattle, WA, USA

Sci Transl Med 2012;4:137ra176

Background: Analysis of cell-free fetal DNA in maternal plasma holds promise for the development of non-invasive prenatal genetic diagnostics. Previous studies have been restricted to detection of fetal trisomies, to specific paternally inherited mutations, or to genotyping common polymorphisms using material obtained invasively, for example through chorionic villus sampling.

Methods: The authors combined genome sequencing of two parents, genome-wide maternal haplotyping, and deep sequencing of maternal plasma DNA to non-invasively determine the genome sequence of a human fetus at 18.5 weeks of gestation.

Results: Inheritance was predicted at 2.8×10^6 parental heterozygous sites with 98.1% accuracy. Furthermore, 39 of 44 de novo point mutations in the fetal genome were detected, albeit with limited specificity. Subsampling these data and analyzing a second family trio by the same approach indicate that parental haplotype blocks of ~300 kilobase pairs combined with shallow sequencing of maternal plasma DNA is sufficient to substantially determine the inherited complement of a fetal genome. However, ultradeep sequencing of maternal plasma DNA is necessary for the practical detection of fetal de novo mutations genome-wide.

Conclusions: Although technical and analytical challenges remain, they anticipate that non-invasive analysis of inherited variation and de novo mutations in fetal genomes will facilitate prenatal diagnosis of both recessive and dominant mendelian disorders.

Parents' blood and saliva were used to sequence the entire genome of a fetus at 18 weeks' gestation, which opens the possibility to non-invasively scan the whole fetal genome for all known single-gene disorders. For exome or genome analyses, 39 de novo (non-inherited) mutations were discovered along with 25 million possible new mutations or false positives. If this information were shared with the parents, would they elect to carry on or terminate this pregnancy?

New clinical tools: Bacterial genomics

Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak

Koser CU, Holden MT, Ellington MJ, Cartwright EJ, Brown NM, Ogilvy-Stuart AL, Hsu LY, Chewapreecha C, Croucher NJ, Harris SR, Sanders M, Enright MC, Dougan G, Bentley SD, Parkhill J, Fraser LJ, Betley JR,

Schulz-Trieglaff OB, Smith GP, Peacock SJ

University of Cambridge, Cambridge, UK

N Engl J Med 2012;366:2267–2275

Background: Isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) belonging to a single lineage are often indistinguishable by means of current antimicrobial phenotyping and limited genotyping techniques. High-throughput sequencing of bacterial genomes may provide improved resolution to define transmission pathways and characterize outbreaks.

Methods: The authors investigated a putative MRSA outbreak in a neonatal intensive care unit. By using rapid high-throughput sequencing technology with a short turnaround time, the authors retrospectively sequenced the DNA from seven isolates associated with the outbreak and another seven MRSA isolates associated with carriage of MRSA or bacteremia in the same hospital.

Results: The authors constructed a phylogenetic tree by comparing single-nucleotide polymorphisms (SNPs) in the core genome to a reference genome (an epidemic MRSA clone, EMRSA-15 (sequence type

22)). This revealed a distinct cluster of outbreak isolates and clear separation between these and the non-outbreak isolates. A previously missed transmission event was detected between 2 patients with bacteremia who were not part of the outbreak. An artificial 'resistome' of antibiotic-resistance genes was created and was shown to predict results of phenotypic antibiotic susceptibility testing; a 'toxome' consisting of toxin genes was also identified. One outbreak isolate had a hypermutator phenotype with a higher number of SNPs than the other outbreak isolates, highlighting the difficulty of imposing a simple threshold for the number of SNPs between isolates to decide whether they are part of a recent transmission chain.

Conclusions: Whole-genome sequencing can provide clinically relevant data within a time frame that is sufficiently short to influence patient care. The need for automated data interpretation and the provision of clinically meaningful reports are remaining hurdles to full clinical implementation.

Bacterial genomics is leading the inevitable advent of genomics technology as a clinical tool in our daily practice. The considerably smaller size of the average bacterial genome, compared to the human genome, means that the time-scales and costs of whole-genome sequencing using currently available platforms are already appropriate to clinical needs. After extracting DNA from an overnight culture, it took the authors only 1.5 days to generate whole-genome sequence data. With faster protocols, the time could be reduced to less than 1 day. In a follow-up paper, the authors also described how this technique was further applied to a series of seemingly unrelated MRSA outbreaks in the same UK special care baby unit (SCBU) [1]. They showed how MRSA transmission occurred within the SCBU, between mothers on a postnatal ward, and in the community. Importantly, it identified and confirmed that MRSA carriage by a single staff member had allowed the outbreak to persist during periods without known infection on the SCBU and after a deep clean. The authors argue that the cost of rapid whole-genome sequencing of one MRSA isolate, currently only GBP 95 (far less than the current GBP ~5,000 for a human whole-genome sequence), is already cost-effective compared to the estimated health-care costs of an MRSA outbreak, at least GBP 10,000.

In addition to benefits for accurate infection control, identification of antibiotic-resistance genes could potentially guide antibiotic therapy, well before traditional antibiotic phenotyping results are available, and could also inform the understanding of new drug-resistance mechanisms. The authors predict that whole-genome sequencing will become a standard tool for infection control both within and outside hospitals.

New treatment: Donor feces

Duodenal infusion of donor feces for recurrent *Clostridium difficile*

Van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, Visser CE, Kuijper EJ, Barteldsman JF, Tijssen JG, Speelman P, Dijkgraaf MG, Keller JJ

Department of Internal Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands
N Engl J Med 2013;368:407–415

Background: Recurrent *Clostridium difficile* infection is difficult to treat; failure rates for antibiotic therapy are high. The authors studied the effect of duodenal infusion of donor feces in patients with recurrent *C. difficile* infection.

Methods: The authors randomly assigned patients to receive one of three therapies: (i) initial vancomycin therapy (500 mg orally QDS for 4 days) followed by bowel lavage and subsequent infusion of a solution of donor feces through a nasoduodenal tube; (ii) standard vancomycin therapy (500 mg orally QDS for 14 days), or (iii) standard vancomycin therapy plus bowel lavage. The primary endpoint was the resolution of diarrhea associated with *C. difficile* infection without relapse after 10 weeks.

Results: The study was stopped after an interim analysis. Of 16 patients in the infusion group, 13 (81%) had resolution of *C. difficile*-associated diarrhea after the first infusion. The 3 remaining patients received a second infusion with feces from a different donor, with resolution in 2 patients. In contrast, resolution of *C. difficile* infection occurred in only 4 of 13 patients (31%) receiving vancomycin alone, and in only 3 of 13 patients (23%) receiving vancomycin plus bowel lavage ($p < 0.001$ for both com-

parisons with the infusion group). No significant differences in adverse events among the three study groups were observed except for mild diarrhea and abdominal cramping in the infusion group on the infusion day. After infusion of donor feces, patients showed increased fecal bacterial diversity, similar to that in healthy donors, with an increase in Bacteroidetes species and *Clostridium* clusters IV and XIVa and a decrease in Proteobacteria species.

Conclusions: Infusion of donor feces was more effective than vancomycin in patients with recurrent *C. difficile* infection.

While for some the thought of donor feces might seem to be repulsive, there are apparently more than 500 published cases of its use worldwide covering a span of 50 years – but this is the first time that its efficacy has been robustly demonstrated in a clinical trial. The results are indeed convincing: donor feces cured 15/16 (94%), mainly elderly, patients compared to only 7/26 (27%) on standard vancomycin therapy. Furthermore, 18 of those who relapsed in the control groups later received off-protocol donor-feces infusions and 15 (83%) of these patients were also cured. The mechanism appears to be the re-establishment of a normal gut microbiota as a host defense against *C. difficile*. As evidence increases for the role of the gut microbiota in the pathogenesis of other non-communicable diseases, including obesity and type 2 diabetes (see paper below), the potential benefits of healthy donor feces are wide!

New mechanisms: Unhealthy feces

A metagenome-wide association study of gut microbiota in type 2 diabetes

Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J
BGI-Shenzhen, Shenzhen, China
Nature 2012;490:55–60

Background: Characterization of gut microbiota has become a major research approach to understanding the pathogenesis of human disease, including type 2 diabetes (T2D), the most prevalent endocrine disease worldwide.

Methods: The authors performed an analysis of gut microbial content in patients with T2D, by developing a protocol for a metagenome-wide association study (MGWAS) and implementing a two-stage MGWAS based on deep shotgun sequencing of the gut microbial DNA of 345 Chinese T2D patients and non-diabetic controls. They established the concept of a metagenomic linkage group, enabling taxonomic species-level analyses.

Results and Conclusions: Approximately 60,000 T2D-associated markers were identified and validated. MGWAS analysis showed that patients with T2D were characterized by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens, as well as an enrichment of other microbial functions conferring sulfate reduction and oxidative stress resistance. An analysis of 23 additional individuals demonstrated that these gut microbial markers might be useful for classifying T2D.

Gut microbiota has been implicated in the pathogenesis of non-communicable, as well as communicable disease, including inflammatory bowel disease and obesity. Previous studies had also shown differences in gut microbiota in adults with and without T2D [2], but the current study is at least 10-fold larger, which allowed them to identify a large number of metagenomic differences between cases and controls. Overall, 3.8% of gut microbial genes showed differences in abundance between T2D cases and controls, indicating modest dysbiosis, i.e. disruption of the balance of the normal microbiota, less than in patients with inflammatory bowel disease. The authors developed and performed 'metagenomic linkage groups' to characterize the many associated genes by taxonomy and

abundance, and to allow assessment of the functional differences. Particularly interesting pathways included butyrate biosynthesis and sulfate reduction. Butyrate is a by-product of the metabolism of plant-based foods, and butyrate-producing bacteria seem to be protective against several diseases. Furthermore, an identified subset of 50 gut microbial gene markers allowed good discrimination between an independent sample of cases and controls (area under the receiver operating characteristic (ROC) curve was 0.81).

Food for thought: How old was your father?

Rate of de novo mutations and the importance of father's age to disease risk

Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WS, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OT, Thorsteinsdottir U, Stefansson K
deCODE Genetics, Reykjavik, Iceland
kong@decode.is
Nature 2012;488:471–475

Background: Mutations generate genetic diversity and provide a substrate for selection. The rate of de novo mutations is therefore of major importance to evolution.

Methods: The authors performed a study of genome-wide mutation rates by sequencing the entire genomes of 78 Icelandic parent-offspring trios at high coverage.

Results: The average father's age was 29.7, and the average de novo mutation rate was 1.20×10^{-8} mutations per nucleotide per generation. Most notably, the diversity in mutation rate of single nucleotide polymorphisms was dominated by the age of the father at time of conception of the proband. The effect size equates to about two additional mutations in an individual for every 1-year older age of the father. An exponential model estimates that the number of paternal mutations doubles every 16.5 years. After accounting for random Poisson variation, father's age is estimated to explain nearly all of the remaining variation in the de novo mutation counts.

Conclusions: These observations shed light on the importance of the father's age on the risk of sporadic genetic diseases, including schizophrenia and autism.

The normal processes of DNA replication, repair and recombination are generally highly accurate; base substitutions occur at an average rate of only 1 mutation for every 100 million bases per generation. However, given the huge size of the genome (and the high current average paternal age), the average child born today carries around 70 de novo mutations, 4-fold more from their father than their mother. The association with paternal, but not maternal, age is to be expected when you consider that spermatozoa are continually produced (spermatogonial stem cells replicate around 23 times per year) whereas the number of oocytes is fixed at birth.

Maternal age, of course, has long been associated with increasing risk of chromosomal aneuploidy, such as Down, Edwards and Patau syndromes. By contrast, the relevance of increasing paternal mutation rates with age on disease risks is only recently being appreciated. While, fortunately, the large majority of de novo mutations do not result in functional or even deleterious changes, a small proportion of such de novo mutations likely contribute to the epidemiological associations between older father's age and higher risks for autism and schizophrenia. Indeed, a trio of papers published in *Nature* in April 2012 described a large haul of de novo mutations in hundreds of genes in patients with autistic spectrum disorder identified by whole-exome sequencing in probands and their parents [3–5].

Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis

Cross-Disorder Group of the Psychiatric Genomics Consortium, Smoller JW, Craddock N, Kendler K, Lee PH, Neale BM, Nurnberger Jr, Ripke S, Santangelo S, Sullivan PF
Lancet 2013;381:1371–1379

Background: Findings from family and twin studies suggest that genetic contributions to psychiatric disorders do not in all cases map to present diagnostic categories. The Psychiatric Genomics Consortium aimed to identify the specific variants and in particular the genetic overlap for five disorders: autism spectrum disorder, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia.

Methods: The authors analyzed genome-wide single-nucleotide polymorphism (SNP) data for the five disorders in 33,332 cases and 27,888 controls of European ancestry. They aimed to identify new specific allelic associations with a best-fitting model of multiple disorders, they examined cross-disorder effects of genome-wide significant loci previously identified for bipolar disorder and schizophrenia, and used polygenic risk-score analysis to examine such effects from a broader set of common variants. Pathway analyses were used to establish the biological associations underlying. Enrichment analysis of expression quantitative trait loci (eQTL) data were used to assess whether SNPs with cross-disorder association were enriched for regulatory SNPs in post-mortem brain-tissue samples.

Results: SNPs at four loci surpassed the cut-off for genome-wide significance ($p < 5 \times 10^{-8}$) in the primary analysis: regions on chromosomes 3p21 and 10q24, and SNPs within two L-type voltage-gated calcium channel subunits, CACNA1C and CACNB2. Model selection analysis supported effects of these loci for several disorders. Loci previously associated with bipolar disorder or schizophrenia had variable diagnostic specificity. Polygenic risk scores showed cross-disorder associations, notably between adult-onset disorders. Pathway analysis supported a role for calcium channel signaling genes in all five disorders. Finally, SNPs with evidence of cross-disorder association were enriched for brain eQTL markers.

Conclusions: Specific SNPs are associated with a range of psychiatric disorders of childhood onset or adult onset. In particular, variation in calcium-channel activity genes seems to have pleiotropic effects across a range of psychopathologies. These findings support the goal of moving beyond the current descriptive basis for psychiatric syndromes, and towards a nosology informed by pathogenesis.

DSM-5, the fifth edition of the American Psychiatric Association's classification of mental disorders, was published on May 18, 2013; it serves as the universal authority for the diagnosis of psychiatric disorders. Notable changes include dropping Asperger syndrome; revised naming of gender identity disorder to gender dysphoria, and inclusion of a new gambling disorder. The publication has been much debated, with some arguing that distinctions that are not supported by good evidence. Indeed, such arguments are as long as the history of current psychiatric nosology, which is based on descriptive symptom patterns and illness course. The boundaries between psychiatric disorders are not clear cut, as many clinical features, for example psychosis, mood dysregulation, and cognitive impairments, are common across different diagnostic categories.

In the above paper, the Cross-Disorder Group of the Psychiatric Genomics Consortium aimed to identify the common basis for a range of five seemingly distinct psychiatric disorders. In the largest genome-wide analysis of psychiatric illness to date, they found that both specific genetic variants and polygenic risk scores were indeed associated with a range of disorders. In particular, there was more overlap between the adult-onset disorders (bipolar and major depressive disorder, and schizophrenia), and calcium signaling, appeared to be pivotal in the mechanisms of all these disorders. It is noteworthy that calcium channel antagonists have antidepressant-like effects in mice, and a target of lithium, the inositol-1,4,5-triphosphate receptor, is a fundamental regulator of calcium release from intracellular stores. Controversially, they propose that genetic 'trans-diagnostic' risk factors may confer susceptibility to a common psychiatric endophenotype.

Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict

Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY

Howard Hughes Medical Institute and Immunology Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Cell 2012;150:29–38

Background: Regulatory T (Treg) cells are generated in both the thymus (tTreg) and extrathymic tissues (peripheral, pTreg). Their differentiation and function are controlled by X-chromosome-encoded transcription factor Foxp3, and their deficiency results in fatal autoimmunity.

Methods and Results: The authors demonstrate that an enhancer of Foxp3, conserved non-coding sequence 1 (CNS1), is present only in placental mammals and is essential for pTreg but not tTreg generation. CNS1 is largely composed of mammalian-wide interspersed repeats (MIR) that have undergone retrotransposition during early mammalian radiation. During pregnancy, pTreg cells specific to a model paternal alloantigen were generated in a CNS1-dependent manner and accumulated in the placenta. Furthermore, when mated with allogeneic, but not syngeneic, males, CNS1-deficient females showed increased fetal resorption accompanied by increased immune cell infiltration and defective remodeling of spiral arteries.

Conclusions: The authors suggest that a CNS1-dependent mechanism of extrathymic differentiation of Treg cells emerged during the evolution of placental animals to enable maternal-fetal tolerance.

A remarkable feature of normal pregnancy in humans and other placental mammals is that the mother tolerates the presence of paternal alloantigens without immune rejection. Regulatory T (Treg) cells appear to have a critical role in establishing such immune tolerance, as their numbers increase during pregnancy and women with low numbers of CD25⁺CD4⁺ Treg cells are susceptible to repeated spontaneous abortions and pre-eclampsia [6].

The authors identified CNS1 as a critical enhancer of extrathymic Treg differentiation, which has a critical role in preventing immune cell infiltration and resorption of embryos in allogeneic matings in mice. Analysis of this gene across different species shows that it is present in all placental mammals and absent in non-placental mammals and non-mammals. Even more striking, the gene sequence of CNS1 puts in a family of retrotransposons (jumping genes) that was active during evolution of placental mammals during the Mesozoic era, when dinosaurs flourished and then went extinct, and mammals and birds appeared for the first time. Other retrotransposon-derived genes required for placentation, including Peg10 and Peg11/Rtl1 and syncitin-A and -B, have retroviral origins [7]. The authors propose that retrotransposon-mediated innovations enabled the evolution of placentation and at the same time provided the solution to the consequent maternal-fetal immune conflict. The findings also inform our understanding and future management of recurrent miscarriage, pre-eclampsia and intrauterine growth retardation.

Is it all in the diet?

Hunter-gatherer energetics and human obesity

Pontzer H, Raichlen DA, Wood BM, Mabulla AZ, Racette SB, Marlowe FW

Department of Anthropology, Hunter College, New York, NY, USA

herman.pontzer@hunter.cuny.edu

PLoS One 2012;7:e40503

Background: Western lifestyles differ markedly from those of our hunter-gatherer ancestors, and these differences in diet and activity level are often implicated in the global obesity pandemic. However, few physiological data for hunter-gatherer populations are available to test these models of obesity.

Methods: The authors performed doubly-labeled water measures of total daily energy expenditure (kcal/day) in Hadza hunter-gatherers to test whether foragers expend more energy each day than their Western counterparts.

Results: As expected, physical activity level (PAL) was higher among Hadza foragers than among Westerners. Nonetheless, after controlling for body size, average daily energy expenditure of traditional Hadza foragers was no different than that of Westerners. The metabolic cost of walking ($\text{kcal kg}^{-1} \text{m}^{-1}$) and resting ($\text{kcal kg}^{-1} \text{s}^{-1}$) were also similar among Hadza and Western groups.

Conclusions: The similarity in metabolic rates across a broad range of cultures challenges current models of obesity suggesting that Western lifestyles lead to decreased energy expenditure. The authors hypothesize that human daily energy expenditure may be an evolved physiological trait largely independent of cultural differences.

We gain weight when our food energy intake exceeds total energy expenditure. Rising obesity incidence is thought to result from our lifestyle, where activity levels decreased and energy intake increased as compared to the original conditions under which humans evolved. This study accurately measured daily energy expenditure and physical activity levels in contemporary hunter-gatherers, who seem to represent our Paleolithic ancestors. The Hadza are foragers of Northern Tanzania who live as similar as it goes to Paleolithic. Do they really expend more energy each day than subjects in market and farming economies? The results show that the Hadza do indeed engage in high levels of physical activity, reflecting their dependence on wild foods. However, taking their smaller body sizes into account, their total energy expenditure (TEE) is similar to Westerners and others in market economies. Even more intriguingly, variation in adiposity both within and between populations was unrelated to physical activity level. Both the Hadza and Westerners utilize only some 10% of TEE for physical activity. Therefore one needs a tremendous increase in physical activity to affect the TEE. If metabolic needs are similar and physical activity counts little, what then makes the difference between them and us, and between overweight and lean? Is it all in the diet?

Difficulties arise when comparing TEE and physical activity energy expenditure across groups with large differences in body size: mean body weight in the Hadza women and men were 43 and 51 kg, respectively, compared to 74 and 81 kg in Westerners. The debate continues.

References

1. Harris SR, Cartwright EJ, Torok ME, et al: Whole-genome sequencing for analysis of an outbreak of methicillin-resistant *Staphylococcus aureus*: a descriptive study. *Lancet Infect Dis* 2013;13:130–136.
2. Larsen N, Vogensen FK, van den Berg FW, et al: Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PloS One* 2010;5:e9085.
3. O’Roak BJ, Vives L, Girirajan S, et al: Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* 2012;485:246–250.
4. Sanders SJ, Murtha MT, Gupta AR, et al: De novo mutations revealed by whole-exome sequencing are strongly associated with autism. *Nature* 2012;485:237–241.
5. Neale BM, Kou Y, Liu L, et al: Patterns and rates of exonic de novo mutations in autism spectrum disorders. *Nature* 2012;485:242–245.
6. Winger EE, Reed JL: Low circulating CD4⁺ CD25⁺ Foxp3⁺ T-regulatory cell levels predict miscarriage risk in newly pregnant women with a history of failure. *Am J Reprod Immunol* 2011;66:320–328.
7. Cornelis G, Heidmann O, Degrelle SA, et al: Captured retroviral envelope syncytin gene associated with the unique placental structure of higher ruminants. *Proc Natl Acad Sci USA* 2013;110:E828–E837.