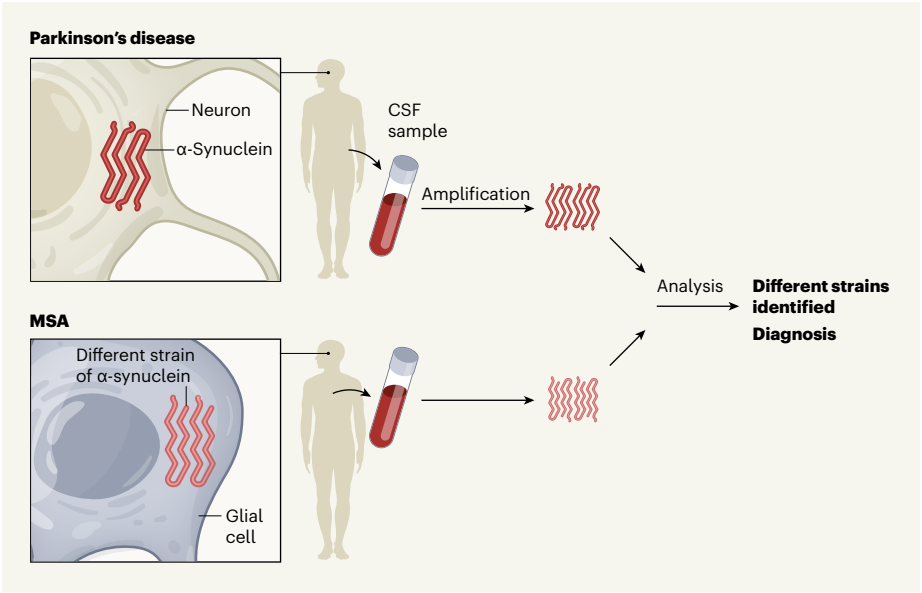

Figure 1 | Different structures for the α-synuclein protein. Two neurodegenerative disorders, Parkinson's disease and multiple system atrophy (MSA), involve aggregates of α-synuclein, which are found in neurons and neuron-supporting glial cells, respectively. Shahnawaz *et al.*[1] have demonstrated that α-synuclein adopts different structures in each disease, indicating that the structure of the protein might contribute to the distinct nature of each disorder. The group extracted tiny amounts of α-synuclein from cerebrospinal fluid (CSF) samples. Protein amplification and analyses revealed different structures for the two samples. These analyses were sufficient to discriminate between the diseases in around 95% of the 200 people studied.

from distinct environmental conditions. For example, different α-synuclein polymorphs arise depending on whether the protein is kept in a phosphate-containing or phosphate-free buffer[9]. *In vivo*, α-synuclein is exposed to several environments. Indeed, the neurons that degenerate in Parkinson's disease and the glia affected in MSA belong to different cell lineages, and have markedly different intracellular environments. In addition, α-synuclein can move between cells, exposing it to both intra- and extracellular environments[2].

The idea of different polymorphs in disease dates back to studies of prion proteins[6] in the 1990s. Much like amyloids, prions aggregate in harmful infectious clumps to cause neurodegenerative conditions such as Creutzfeldt–Jakob disease in humans and scrapie in sheep. Several strains of prion, each adopting a different polymorph, typically coexist in a given sample or organism[7]. The strains have different fitnesses in different environments, which governs their ability to replicate[7] – a phenomenon known as the prion cloud[10].

A corollary of this idea is that if environmental conditions change, the relative abundance of each polymorph might change. This principle also governs the PMCA assay. Under given conditions, the fittest polymorphs should be amplified from a possible mix of pre-existing strains. Indeed, in Shahnawaz and colleagues' experiments, a single distinct polymorph was amplified from Parkinson's disease samples and another from MSA samples.

By contrast, in another recent study that used PMCA, Strohäker and colleagues[11] reported no significant differences between structures of α-synuclein derived from the brains of people who had Parkinson's disease and those with from people with MSA. A possible explanation for this apparent discrepancy is that the two groups used different PMCA protocols. In addition, Strohäker *et al.* used a much smaller group of patients than did Shahnawaz and colleagues. In fact, analysis using nuclear magnetic resonance spectroscopy did indicate distinct structural features in a subset of Strohäker and colleagues' samples.

High-resolution cryo-electron microscopy has been used to demonstrate the existence of distinct disease-specific polymorphs of another neurodegeneration-associated protein, tau, at atomic resolution[8]. A similar approach using samples extracted under mild conditions might give us a clearer picture of the reality for α-synuclein. Taken together with similar observations for Alzheimer's disease[12], our understanding of the structural landscape of amyloid diseases is broadening.

Juan Atilio Gerez and **Roland Riek** are in the Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, ETH-Hönggerberg, 8093 Zurich, Switzerland.
e-mails: juan.gerez@phys.chem.ethz.ch; roland.riek@phys.chem.ethz.ch

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Medical research

Smoke signals in the DNA of normal lung cells

Gerd P. Pfeifer

Healthy cells in smokers' lungs have a high burden of mutations, similar to the mutational profile of lung cancer. Surprisingly, ex-smokers' lungs have a large fraction of healthy cells with nearly normal profiles. **See p.266**

According to the World Health Organization, there are 1.1 billion smokers worldwide and an estimated 1.8 million deaths from lung cancer annually. Lung cancer caused by smoking can take decades to arise, and smokers have up to a 30-fold higher risk of developing the disease than do non-smokers[1]. Carcinogenic components of tobacco smoke promote lung cancer by causing DNA damage that can lead to mutations through known mechanisms, but what the initial consequences of smoking are for healthy lung cells is poorly understood. On page 266, Yoshida *et al.*[2] report the mutational profiles of 632 healthy lung cells obtained from whole-genome sequencing of biopsied tissue from 16 individuals: children, adults, non-smokers, current smokers and ex-smokers. The authors analysed the frequency and properties of the mutations present, how they differed according to age

and smoking status, and how these mutations related to those found in a type of lung cancer called squamous-cell carcinoma.

The authors dissociated cells from lung tissue (Fig. 1) and isolated a type of epithelial cell called a basal cell (which can self-renew). Growing single cells into cellular colonies allowed the authors to determine the DNA sequence of the given original cell. A potential caveat of the study is that, although the authors obtained the genome sequences of hundreds of single cells, the number of individuals with each different smoking status was relatively small. The authors report that the number of single nucleotide (point) mutations increased with age – for each extra year of life, about 22 additional such mutations were found per cell.

However, being a former smoker added another 2,330, and being a current smoker added 5,300 point mutations per cell on average, confirming the mutational potency of smoking. Smokers' genomes also had extensive examples of other types of alteration, such as insertion or deletion mutations. The number of mutations in different cells from the same individual could vary by tenfold in smokers, a much higher variability than was found in non-smokers. The stage of the cell cycle at which a cell is exposed to carcinogenic agents might affect how effectively DNA damage is repaired before DNA replication, which could offer an explanation for this high variability.

Yoshida and colleagues examined the mutations in individual cells using previously developed algorithms to focus on all the types of sequence alteration possible (for example, mutation of the DNA base adenine to cytosine, guanine or thymine) and also to assess the bases on either side of a mutated base. Such analysis identifies specific patterns (mutational signatures) that have been used before to characterize the genomes of tumour cells[3].

The authors report that the presence of certain mutational signatures increased with age and did not seem to be affected by smoking. These included a signature attributed to natural processes whereby the loss of an amino group in a modified cytosine (termed 5-methylcytosine) changes the base to a thymine. The most common mutational signature in all the samples was one that is rich in cytosine-to-thymine and thymine-to-cytosine mutations. The presence of this signature increased with age and was more common in people with a history of smoking. The underlying processes driving these mutations are unknown. The most common smoking-dependent signature consisted of guanine-to-thymine mutations, a signature that is characteristic of most smoking-associated lung cancers[4–7].

Lung cancers have some of the highest mutation frequencies of all tumour types[8]; however, it is thought that only a small number of tumour-promoting (driver) mutations need to occur in a single cell to kick off malignant growth. Given the high mutational burden and the specific smoking-associated mutational signatures found in smokers' healthy epithelial cells, Yoshida and colleagues examined whether these mutations affected crucial genes that are relevant for cancer growth.

Indeed, they found cells that had acquired mutations in genes, including *TP53* and *NOTCH1*, that are driver mutations in squamous-cell carcinomas. These driver mutations were more common in the lung cells of smokers than in those of non-smokers. Some cells even had as many as three driver mutations. However, we do not know how many of these mutations (and in what combination) are required for human lung cancer to develop. Specific *TP53* mutations were found in multiple cells from the same individual, suggesting that these mutations occur early, that cells with the mutation proliferate, or both – similar to what has been observed for sun-exposed healthy human skin[9].

The higher risk of lung cancer in ex-smokers compared with non-smokers is reflected in their high mutation burden and the signature of smoking-associated mutations in most of their lung cells (similar to the cellular profile of current smokers). Although ex-smokers have a high risk of developing lung cancer, their risk is reduced compared with that of current smokers, and this lowering depends on the length of time of smoking cessation[1]. Why this is the case has been hard to explain. However, perhaps the most surprising result of Yoshida and colleagues' work might offer a clue: in 5 out of 6 ex-smokers, 20–50% of the cells had a low mutation burden that was similar to the profile of non-smokers of the same age range (Fig. 1).

These near-normal cells in ex-smokers had a low frequency of smoking-dependent mutational signatures. Moreover, compared with the ex-smokers' highly mutated cells, these near-normal cells had longer versions of DNA structures called telomeres, which are found at the ends of chromosomes. Telomere length shortens with each cell division; thus, long telomeres suggest that these cells had not undergone many divisions. The authors speculate that these cells might have arisen comparatively recently from divisions of proposed previously dormant (quiescent) stem cells. However, whether such cells exist in human lungs is unknown.

DNA damage can generate a mutation during DNA replication. Therefore, if a population of non-dividing stem cells exists in the human lung, even if exposed to carcinogenic agents, perhaps such cells might avoid incurring mutations if DNA damage is eventually repaired in the absence of division. But the lack of knowledge about these proposed long-lived stem cells and information about the longevity of the different cell types in the human lung make it difficult to explain what occurred in

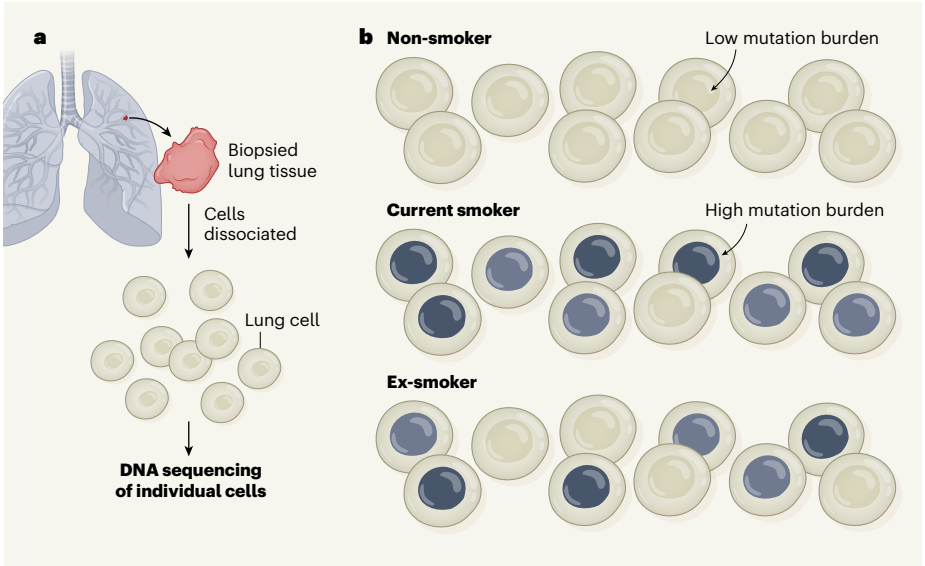

Figure 1 | Mutational burdens in normal human lung cells. Yoshida *et al.*[2] analysed the pattern of mutations in healthy lung tissue in non-smokers, current smokers and ex-smokers. **a**, Using biopsied lung tissue, the authors determined whole-genome sequences corresponding to single cells. **b**, The cells of the non-smoking individuals had few mutations. By contrast, current smokers had a high proportion of cells with a large number of mutations (grey; darker colour indicates more mutations), and many of these mutations were of a type predominantly found in smokers. Compared with non-smokers, smokers also had greater variability in the mutational load between the different cells of a given individual. Surprisingly, the authors found that five out of six ex-smokers had a substantial fraction (20–50%) of cells that had low numbers of mutations and had hardly any smoking-associated mutational signatures. How these cells arise is a mystery – Yoshida *et al.* speculate that they are generated from a population of as-yet-unknown stem cells.

these ex-smokers' cells with few mutations.

Why do ex-smokers still have a substantial fraction of highly mutated cells that can proliferate, at least when grown *in vitro*? Any short-lived cells that were exposed to carcinogens during their proliferation should have vanished many years after the cessation of smoking. This raises the question of whether there are long-lived differentiated cells in the lung that carry a high mutational burden, and whether these cells can resume proliferation, perhaps because of the plasticity (the ability to change cellular identity) of lung cells[10]. A future challenge will be to understand the cell biology of the mechanisms underlying these observations. Perhaps one day it will be possible to develop ways to boost the population of lung cells with few mutations in ex-smokers.

Yoshida and colleagues' study has broadened our understanding of the effects of tobacco smoke on normal epithelial cells in the human lung. It has shed light on how the protective effect of smoking cessation plays out at the molecular level in human lung tissue and raises many interesting questions worthy of future investigation.

Gerd P. Pfeifer is at the Center for Epigenetics, Van Andel Institute, Grand Rapids, Michigan 49503, USA.
e-mail: gerd.pfeifer@vai.org

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Forum: Mental health

Digital technology under scrutiny

Does time spent using digital technology and social media have an adverse effect on mental health, especially that of adolescents? Here, two scientists discuss the question, and how digital devices might be used to improve well-being.

The topic in brief

- **There is an ongoing debate about whether social media and the use of digital devices are detrimental to mental health.**
- **Adolescents tend to be heavy users of these devices, and especially of social media.**
- **Rates of teenage depression began to rise around 2012, when adolescent use of social media became common (Fig. 1).**
- **Some evidence indicates that frequent users of social media have higher rates of depression and anxiety than do light users.**
- **But perhaps digital devices could provide a way of gathering data about mental health in a systematic way, and make interventions more timely.**

Jonathan Haidt
A guilty verdict

A sudden increase in the rates of depression, anxiety and self-harm was seen in adolescents — particularly girls — in the United States and the United Kingdom around 2012 or 2013 (see go.nature.com/2up38hw). Only one suspect was in the right place at the right time to account for this sudden change: social media. Its use by teenagers increased most quickly between 2009 and 2011, by which point two-thirds of 15–17-year-olds were using it on a daily basis[1]. Some researchers defend social media, arguing that there is only circumstantial evidence for its role in mental-health problems[2,3]. And, indeed, several studies[2,3] show that there is only a small correlation between time spent on screens and bad mental-health outcomes. However, I present three arguments against this defence.

First, the papers that report small or null effects usually focus on 'screen time', but it is not films or video chats with friends that damage mental health. When research papers allow us to zoom in on social media, rather than looking at screen time as a whole, the correlations with depression are larger, and they are larger still when we look specifically at girls (go.nature.com/2u74der). The sex difference is robust, and there are several likely causes for it. Girls use social media much more than do boys (who, in turn, spend more of their time gaming). And, for girls more than boys, social life and status tend to revolve around intimacy and inclusion versus exclusion[4], making them more vulnerable to both the 'fear of missing out' and the relational aggression that social media facilitates.

Second, although correlational studies can provide only circumstantial evidence, most of the experiments published in recent years have found evidence of causation (go.nature.com/2u74der). In these studies, people are randomly assigned to groups that are asked to continue using social media or to reduce their use substantially. After a few weeks, people who reduce their use generally report an improvement in mood or a reduction in loneliness or symptoms of depression.

Third, many researchers seem to be thinking about social media as if it were sugar: safe in small to moderate quantities, and harmful only if teenagers consume large quantities. But, unlike sugar, social media does not act just on those who consume it. It has radically transformed the nature of peer relationships, family relationships and daily activities[5]. When most of the 11-year-olds in a class are on Instagram (as was the case in my son's school), there can be pervasive effects on everyone. Children who opt out can find themselves isolated. A simple dose–response model cannot capture the full effects of social media, yet nearly all of the debate among researchers so far has been over the size of the dose–response effect. To cite just one suggestive finding of what lies beyond that model: network effects for depression and anxiety are large, and bad mental health spreads more contagiously between women than between men[6].

In conclusion, digital media in general undoubtedly has many beneficial uses, including the treatment of mental illness. But if you focus on social media, you'll find stronger evidence of harm, and less exculpatory evidence, especially for its millions of under-age users.

What should we do while researchers hash out the meaning of these conflicting findings? I would urge a focus on middle schools (roughly 11–13-year-olds in the United States), both for researchers and policymakers. Any US state could quickly conduct an informative experiment beginning this September: randomly assign a portion of school districts to ban smartphone access for students in