to the potentially fatal immune response observed in people who develop severe COVID-19 symptoms. As a result, a haplotype that at times in our past might have been beneficial for survival could now be having an adverse effect.

Despite the correlation between this risk haplotype and clinical outcomes, genetics alone do not determine a person's risk of developing severe COVID-19. Our genes and their origins clearly influence the development and progression of COVID-19 (and other infectious diseases), but environmental factors also have key roles in disease outcomes.

For example, although the Neanderthal-derived risk haplotype is almost completely absent in people with African ancestry, this population has a higher COVID-19 mortality rate than do people of other ethnic backgrounds, even after adjusting for geography and socio-economic factors (see go.nature.com/3jcxezx ('Demographics' tab) and go.nature.com/2h4qfqu, for example). Social inequality and its repercussions seem likely to account for a larger proportion of the risk of COVID-19 death than does Neanderthal-derived DNA.

It is fascinating to think that our ancestor's genetic legacy might be playing a part in the current pandemic. However, the underlying impact of the inherited DNA on the body's response to the virus is unclear. Ongoing global efforts to study associations between our genetics and COVID-19 by analysing more individuals from diverse populations, such as that being undertaken by the COVID-19 Host Genetics Initiative (www.covid19hg.org), will help us to develop a better understanding of the disease's aetiology. It is important to acknowledge that, although genes involved in the COVID-19 response might be inherited, social factors and behaviours (such as social distancing and mask wearing) are in our control, and can effectively reduce the risk of infection.

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Biochemistry

Isoforms combine for diverse signalling

Joshua C. Snyder & Sudarshan Rajagopal

Many receptor proteins of the GPCR family exist in multiple isoforms. A comprehensive analysis of different combinations of GPCR isoforms that produce diverse signalling patterns in cells has implications for drug development. **See p.650**

With more than 800 members[1], the G-protein-coupled receptor (GPCR) superfamily is the largest family of cell-surface receptor proteins in humans. GPCRs trigger intracellular signalling pathways in response to activation by extracellular factors. In doing so, they determine how a cell responds to and interacts with its environment, thereby influencing nearly every aspect of physiology. As such, they are excellent drug targets – at least 475 drugs approved by the US Food and Drug Administration (FDA) are aimed at GPCRs[2]. But many GPCRs exist in multiple isoforms, or variants, complicating attempts to find drugs that can bind to them. On page 650, Marti-Solano *et al.*[3] describe a catalogue of the structure and expression of GPCR isoforms in humans. This resource has been added to a GPCR database, called GPCRdb, and is already openly available to the scientific community[4] (https://gpcrdb.org/protein/isoforms).

One common hurdle when attempting to design drugs that control GPCR signalling is that the same GPCR can activate multiple intracellular signalling pathways[5]. Pharmacologically altering the receptor's activity can therefore lead to unforeseen side effects. Drugs called biased agonists that target just one pathway downstream of GPCRs have shown great promise[6,7]. However, they are effective in only some cases – perhaps because the genes that encode GPCRs can be processed in different ways during transcription, producing multiple versions of the final messenger RNA, called splice variants. Through this splicing mechanism, specific domains can be excluded from a GPCR or atypical ones added, producing a range of isoforms. Each one might preferentially activate alternative downstream signalling pathways. So far, our understanding of this key aspect of GPCR biology has been limited to studies of a few isoforms in unnatural settings[8,9].

Marti-Solano and colleagues set out to determine how the presence of various isoforms affects the signalling of around 350 GPCRs across tissues of the human body. First, they made use of information about GPCR structures and DNA sequences from GPCRdb to help them identify candidate GPCRs in a database called GTex – a catalogue of gene expression in human tissues. This produced a list of 625 GPCR isoforms, with 38% of GPCRs having more than one.

The group then systematically organized these GPCR isoforms according to their topology. They developed a set of 'structural fingerprints' for GPCR isoforms, based on the specific extracellular, intracellular and transmembrane domains present in each one (Fig. 1a). The most common structural fingerprints preserved GPCR topology, and the most frequent changes were seen only in the protein's extracellular amino terminus or intracellular carboxy terminus. The N-terminal alterations typically caused changes in the binding of ligand molecules or efficacy. By contrast, C-terminal alterations led to changes in the ability of the receptor to couple with other receptor monomers, or in alterations in the internalization or transport of receptors through the cell inside vesicles – all of which are key to downstream signalling.

The authors also found a few truncated isoforms, in which transmembrane domains were eliminated. They propose that these decrease receptor signalling. The truncated isoforms might be expressed only inside the cell, where they bind to more-complete versions – isoforms internalized in this way are unable to signal.

Next, to model the potential tissue-specific effects of different isoforms, Marti-Solano *et al.* generated tissue-expression signatures – maps of the expression of each isoform for each receptor across 30 tissues. This revealed different combinations across tissues. The authors confirmed that co-expressing various combinations of isoforms of a given receptor in cells in culture resulted in different patterns of downstream signalling (Fig. 1b). It is not surprising that isoforms have different signalling properties. Nonetheless, the demonstration that co-expression of different isoforms alters

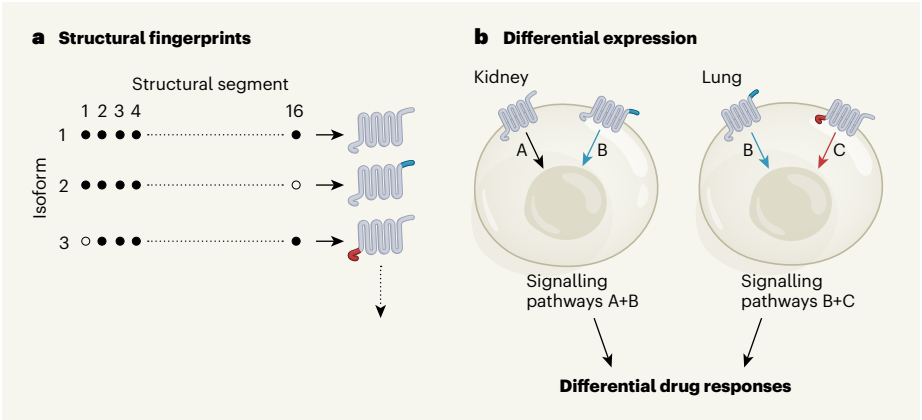

Figure 1 | Cataloguing G-protein coupled receptor (GPCR) isoforms. Marti-Solano *et al.*[3] analysed 625 GPCR isoforms expressed across 30 human tissues, and show that there is often more than one isoform of the same receptor in a tissue. **a**, The authors catalogued structural variation between GPCR isoforms by generating structural fingerprints — descriptions of the structural segments included in each isoform (dark circles indicate segments that are part of a given isoform; empty circles indicate segments that are missing). This simplified schematic shows three isoforms for one imagined GPCR; many more are possible (dashed arrow). **b**, The group shows that these isoforms are expressed in different combinations across tissues. Each combination might activate different downstream signalling pathways, and so respond differently to drugs.

signalling suggests a broad mechanism for generating 'systems bias'[10], in which various tissue-expression signatures promote differential activation of intracellular signalling pathways.

The authors next checked that the tissue-expression signatures they observed truly reflected co-expression of multiple isoforms of a receptor, rather than expression of different isoforms in different cell types within a tissue. They analysed isoform-level expression in various cell lines, as well as data from single-cell RNA sequencing. These assays confirmed that single cells expressed multiple receptor isoforms.

Lastly, Marti-Solano *et al.* showed that 42% of the 111 GPCRs that are targets of FDA-approved drugs had more than one isoform — and that in many cases each of the isoforms for a given receptor has a different tissue distribution. The authors also found that specific single-nucleotide DNA mutations in some were associated with disease. This finding suggests that isoform-selective drugs might be useful for treating human diseases. The search for these drugs recalls the long and continuing process of developing subtype-selective drugs for many GPCRs[11] (drugs that modulate just one of the 13 GPCRs activated by the chemical serotonin, for instance). The current finding indicates that drugs might need to be both subtype- and isoform-selective. Time will tell whether thoughtful design of isoform-selective drugs will lead to increased specificity and fewer off-target effects than occur with current drugs.

It is important to note the limitations inherent in this study. Isoform expression could be assessed only at the level of gene expression, whereas the gold standard in receptor biology is to measure protein expression using an approach called radioligand binding. However, radioligands might not differentiate between receptor isoforms. Furthermore, Marti-Solano *et al.* did analyse mass spectrometry data to confirm protein expression for some isoforms.

Another caveat is that the authors' experiments on how isoforms act in combination involved expressing the proteins in an atypical setting, in cells in culture. However, the biology of these isoforms is complex and cell-specific. Their behaviour might depend on spatial localization of receptors, on cell-specific cofactors, or on the isoforms' ability to control cell-intrinsic responses to an external microenvironment. As such, an *in vitro* setting might not fully reveal how each isoform would act *in vivo*.

"Time will tell whether thoughtful design of isoform-selective drugs will lead to increased specificity and fewer off-target effects."

It will also be of interest to determine whether plasticity in isoform expression serves as a mechanism by which to dynamically regulate system-level responses. Could tissue-expression signatures change over time or in response to signals from other regions of the body, enabling a tissue to respond to the same signal in different ways under different conditions?

A related avenue for future research will be to systematically determine how splicing affects the expression and activity of the protein ligands that bind to GPCRs. New ligand isoforms can arise in cancer, as a result of gene fusions[12]. For example, the expression of these 'oncogenic fusion ligands' leads to changes in the stem-cell microenvironment of the colon that enable the spread of precancerous stem cells[13]. It seems likely that splice isoforms of GPCRs could alter system-level responses in disease, separate from or together with ligand fusions. Going forward, the same possibility could be investigated for all transmembrane receptors, because biased signalling has also been described for receptor tyrosine kinases[14] — these receptors also have a range of isoforms that have roles in health and disease. For example, splice isoforms of the tyrosine kinase ERBB2 are drivers of breast and lung cancers[15,16].

Finally, we need to consider whether we should update our approach to GPCR drug development. The current study clearly indicates that tissue-specific expression of receptor isoforms could complicate attempts to target a given GPCR. This is especially true when trying to design a biased agonist that blocks only one downstream signalling pathway — such drugs might have different effects in different tissues. A better understanding of the system bias induced by different isoform combinations will be needed to overcome this obstacle. Experiments should move beyond cell lines to analyse systems bias in cells taken directly from tissues, or *in vivo*. Marti-Solano and colleagues' valuable resource will be essential for informing such studies.

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