

Transcriptional Dynamics at Brain Enhancers: from Functional Specialization to Neurodegeneration

Marit W. Vermunt¹ · Menno P. Creyghton¹

Published online: 14 September 2016

© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract Over the last decade, the noncoding part of the genome has been shown to harbour thousands of *cis*-regulatory elements, such as enhancers, that activate well-defined gene expression programs. Driven by the development of numerous techniques, many of these elements are now identified in multiple tissues and cell types, and their characteristics as well as importance in development and disease are becoming increasingly clear. Here, we provide an overview of the insights that were gained from the analysis of noncoding gene regulatory elements in the brain and describe their potential contribution to cell type specialization, brain function and neurodegenerative disease.

Keywords Epigenetics · Gene regulation · Enhancer · Common variation · Parkinson's disease · α -synuclein

Introduction

Cell state specification is determined by the tight control of gene expression programs that arise as a result of pre-programmed developmental cascades as well as environmental stimuli [1, 2]. Transcriptional regulation is a dynamic process and is guided by transcription factors that occupy *cis*-regulatory elements (CREs) in noncoding parts of the genome. CREs are short stretches of DNA that contain recognition motifs onto which transcription factors can dock [3].

These in turn recruit cofactors that modify the local chromatin environment and that influence the assembly of a functional transcriptional apparatus at the core promoter of genes.

Our understanding of the basic principles of gene regulation and the role that transcription factors play in this process have increased substantially over the last 10 years [4]. Close to 1400 transcription factors have been identified [5] and recent advances in large-scale sequencing techniques are further expanding our insights of transcriptional regulation by allowing the identification of hundreds of thousands of CREs as well as the networks in which they operate [6]. Small sets of master regulators have emerged as central to the coordination of these transcriptional networks during development [4, 7]. Accordingly, overexpression of such factors can initiate reprogramming of fully differentiated cells towards a different developmental path [8]. Thus, while studying transcription factors and their regulatory networks has provided critical insight into the processes of cell state specification, it has also allowed us to control these states.

Numerous diseases, including most cancers, are caused by aberrant transcriptional regulation which is often the result of mutations in transcription factors or their associated cofactors [4]. Interestingly, a number of diseases have also been linked to mutations within CREs [9–11]. In addition, most single nucleotide polymorphisms (SNPs), associated with a variety of disorders, have been identified in noncoding DNA [12]. Therefore, efforts to understand the genetic and epigenetic basis of pathology have significantly shifted focus from coding sequences (i.e. genes) towards noncoding regulatory elements. This is especially the case for complex diseases such as neuropsychiatric and neurodegenerative disorders, in which much of the underlying heritability has remained elusive [13].

In the current review, we focus on gene expression control in the human brain. We summarise how large-scale identification of *cis*-regulatory DNA is starting to broaden our

This article is part of the Topical Collection on *Genetics*

✉ Menno P. Creyghton
m.creyghton@hubrecht.eu

¹ Hubrecht Institute-KNAW and University Medical Center Utrecht, Uppsalalaan 8, 3584CT Utrecht, The Netherlands

understanding of transcriptional programs in the brain and how this knowledge can be used to uncover gene regulatory alterations that contribute to complex brain diseases.

Unravelling Transcriptional Programs in the Brain

For decades, scientists have been attempting to unravel the transcriptional programs that give rise to the wide variety of neuronal cell types that collectively make up the central nervous system. Several transcription factors were shown to play key roles in neurogenesis and neuronal diversification (for an extensive review see [14]). For instance, the expression of *Dlx1* (distal-less homeobox 1) and *Dlx2* counteracts the expression of *Olig1* (oligodendrocyte transcription factor 1) and *Olig2* to promote interneuron fate over oligodendrocyte specification and vice versa [15, 16]. Furthermore, upregulation of the transcription factor *Pax6* (paired box 6) induces neurogenesis through induction of *Ngn2* (neurogenin 2) [17]. Surprisingly, overexpression of *Pax6* could also induce neurogenesis in post-natal astrocytes *in vitro*, representing one of the earliest examples of lineage conversion directed by a single transcription factor [18]. *Ngn2* as well as *Ascl1* (achaete-scute homolog 1), another key factor in neurogenesis, was also shown to be able to drive neuronal cell fate specification in post-natal astrocytes suggesting that differentiation boundaries could be overcome using specific transcription factors [19]. Following these discoveries, select combinations of transcription factors were identified that, when overexpressed, were able to induce major cell state changes [8]. This included the direct conversion of fibroblasts into neuronal cell types [20], which has now been achieved through overexpression of different combinations of neuronal transcription factors, typically using *Ascl1* as a cornerstone factor (reviewed in [21]). These data demonstrate that transcription factors play a central role in determining cell state specification in the nervous system as well as in controlling the plasticity of these states.

Following the emergence of genome-scale transcriptome analyses, spatio-temporal gene expression programs in the brain are now rapidly being elucidated. Large consortia, including the Allen Institute for Brain Science and *BrainSpan*, have collected gene expression data in murine and human tissues at different developmental stages as well as in brain tissue from humans suffering from neurological disorders [22, 23]. Furthermore, co-expression analysis of these types of data has revealed a hierarchical structure of networks in which certain transcription factors present as central (hub) genes that modulate the expression of other genes [22, 24, 25]. For instance, *TBR1* (T-brain 1) and *EMX2* (empty spiracles homeobox 2) have emerged as hub regulators in the adult human brain [25] and are well-known cortical transcription factors involved in state specification of cortical

progenitors and adult neurons [26]. While these analyses are starting to reveal the hierarchical structure of gene regulatory networks, a full grasp of their complexity can only be achieved when combined with intricate knowledge of the underlying CREs to which these transcription factors bind. The latter analysis has until recent years been lagging behind.

Characteristics of *cis*-Regulatory DNA

CREs are short stretches of noncoding DNA, typically 200–500 base pairs in length, that contain sequence motifs that are recognized and bound by transcription factors (Fig. 1a) [27, 28]. The spacing, location and sequence content of these binding motifs can be either very relaxed or tightly determined depending on which enhancer is assayed [29]. The number of potential binding motifs within the genome for a given transcription factor typically outpaces the number of actual binding events by an order of magnitude [30]. This is primarily explained by the combinatorial nature of transcription factor binding [30–32]. Typically, a hierarchical sequence of binding events will start with the docking of so-called pioneer factors that promote the accessibility of DNA to other proteins [32–34]. This will stabilize a core complex that in turn recruits other, more ubiquitously expressed, cofactors through protein-protein interactions [35]. These cofactors can further modify the local chromatin environment by adding defined epigenetic modifications to histone tails, thus creating additional docking sites for proteins and further altering local chromatin state [36]. Alternatively, they may directly influence transcription initiation at the core promoter.

The combined activity of different transcription factors bound to a single element ultimately determines its regulatory capacity as being an activator (enhancer) or repressor (silencer) of gene expression. CREs are considered part of the promoter when located next to a gene's transcriptional start site onto which the RNA polymerase II transcription initiation complex is assembled. However, they can also be situated at large genomic distances (up to 1 million base pairs [9]) and interact with the promoter of their target gene through a process called chromatin looping [37, 38]. Several factors such as CCCTC-binding factor (CTCF), mediator and cohesin are involved in the establishment of these long-range interactions [39–41]. The genomic architecture within a cell's nucleus is further shaped by CTCF boundaries, called insulators, into neighbourhoods in which genes and their CREs are isolated (Fig. 1a) [41–43]. Target gene regulation is often restricted to these domains and dependent on the combined activity of CREs [40]. Genes can also be regulated by multiple enhancers in a modular fashion meaning that separate enhancers support gene expression in a particular anatomical structure or cell type (Fig. 1a) [29, 32]. For

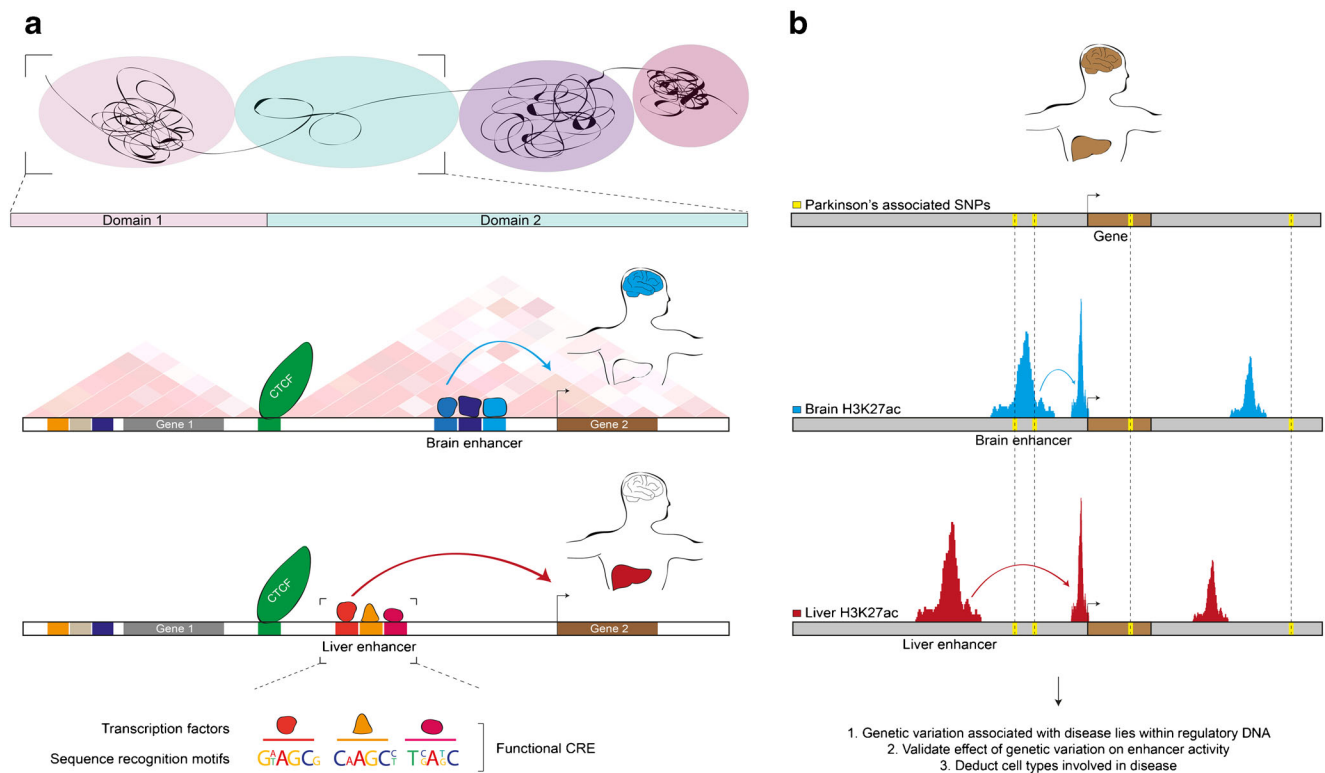


Fig. 1 *Cis*-regulatory elements in gene expression and disease. **a**. The genome is subdivided into chromatin neighbourhoods (*upper panel*). Within domains, delineated by CTCF (*green*) and typically analyzed by chromatin conformation capture techniques (hypothetical Hi-C result shown), enhancers drive target gene expression through long-range interactions with the promoter of their target gene (two examples shown below the domains). Enhancers often regulate genes in a tissue-specific manner as depicted here for the brain (*blue*) and liver (*red*). Functional

CREs comprise stretches of binding motifs that are bound by transcription factors to ultimately determine CRE activity (*bottom*). **b**. The combination of enhancer identification and GWAS studies has revealed that many single nucleotide polymorphisms (*yellow*) lie within *cis*-regulatory DNA (*blue*, H3K27ac in the brain; *red*, H3K27ac in the liver). Variation within CREs can contribute to disease susceptibility and aid in the identification of relevant cell types

instance, different neural enhancers independently regulate the expression of proopiomelanocortin across several neuroanatomical regions [44]. Therefore, sets of transcription factors operate through a number of CREs to orchestrate the activation and repression of genes into well-defined transcriptional programs.

Genome-Wide Annotation of CREs

Early discovery of CREs relied on the careful analysis of a handful of single gene regions [45]. One of the most well-studied examples is the beta-globin locus [46]. Similar efforts have led to the identification of regulatory DNA that acts on the *Pax6* gene in the lens. Later, it became clear that *Pax6* is under the control of multiple enhancers to strictly regulate its expression in the eye, brain and pancreas [47]. This underscored the function of enhancers as modular regulators of gene expression in a cell type-specific manner.

Based on the fact that gene function is typically conserved across species, the assumption that gene regulation would be equally conserved led to the identification of several thousands of predicted enhancer sequences across species using comparative genomics [48, 49]. Typically, half of these highly conserved elements contained measurable enhancer activity when tested in transgenic animals using reporter assays and a significant portion supported expression in the developing nervous system [49, 50]. However, several enhancers that were found by analysing specific genes of interest displayed little evidence of sequence conservation, with some showing no sequence conservation despite being functionally conserved [51, 52]. This raised the question on how much regulatory information was still missing.

The realization that *cis*-regulatory DNA contains specific epigenetic footprints [53, 54], combined with the emergence of large-scale sequencing techniques to measure them genome-wide [53], has significantly propelled our understanding of the regulatory networks that dictate gene expression. For instance, CREs typically reside in open chromatin

and are generally characterized by low nucleosome density in combination with defined histone variants or histone modifications [36]. The accessibility of CREs can be exploited to chart their location at a genome-wide level using a variety of large-scale assays [55–57]. While most of the identified regions are likely enhancers, open chromatin also contains insulators bound by CTCF [58], repressors such as regions occupied by REST (RE1 silencing transcription factor) [59] and potential other regulatory elements [60].

Different types of CREs are associated with distinct histone signatures and transcription factors [36, 61], the location of which can be measured by chromatin immunoprecipitation (ChIP) followed by sequencing. Promoters are mainly marked by histone 3 lysine 4 mono-methylation (H3K4me1) and H3K4me3, while H3K4me1 in the absence of H3K4me3 selectively associates with distal regulatory regions [61]. H3K4me1 may function to protect genomic regions from repressors that bind unmodified H3K4 [62, 63] or alternatively as a docking site for factors that enhance the regulatory potential of the region by altering its accessibility [64]. This signature is found at enhancers that are active, poised or repressed and remains long after activity has ceased [61, 65–67]. The presence of histone 3 lysine 27 acetylation (H3K27ac) is indicative of active promoters as well as enhancers [6, 65, 67–69]. The acetyltransferases p300 and CREB binding protein (CBP) that deposit H3K27ac are similarly used to identify enhancers [70]. Acetylated lysines direct regulatory activity by serving as docking sites for bromodomain containing cofactors [71]. However, they also influence chromatin compaction by attenuating histone-DNA interactions through the neutralization of electrostatic interactions [72]. Acetylation-based enhancer predictions are confirmed in reporter assays in ~70 % of the cases presuming that a substantial fraction of the enhancer sequence is assayed [2, 23, 32, 35]. Furthermore, the discovery rate of enhancers using acetylated lysines is also relatively high [68]. Other assays to assess enhancer activity have been explored and were shown to be indicative of tissue-specific activity as well [73–75]. However, as CRE identification through the use of histone marks is relatively easy and robust, it currently remains the most frequently used method.

Emerging Concepts from Large-Scale Identification of *cis*-Regulatory DNA

The epigenomic analysis of regulatory networks has substantially enhanced our understanding of how CREs operate, how these elements evolved across evolutionary time and according to which rules target genes are specified and controlled. For instance, the majority of distal regulatory elements were found to function as enhancers (over 400,000 predicted), often in a temporal and tissue-specific manner [1, 2, 54, 70]. In contrast, promoters, insulators and the overall topological structure of the genome were mostly found conserved

between cell types [54, 76]. Similar observations were done over evolutionary time. While enhancer activity was shown to be poorly conserved across species, promoters and chromatin architecture were overall similar [77–81]. The fact that enhancers are highly tissue-specific as well as the fact that multiple enhancers can act together on a single gene in a redundant fashion, partially explains this lack of conservation [82, 83]. Furthermore, redundancy in recognition motifs allows enhancers to remain functionally conserved despite a lack of sequence similarity [51, 52, 84–86].

Different types of promoter and enhancer elements were discovered based on their activity. Enhancers can be active (bound by H3K27ac), while they can also exist in a poised state ready to be activated [65, 67]. A poised state is part of the transcriptional program that specifies cell state and gives the cell a set of transcriptional options to deploy rapidly in response to environmental cues. For instance, neurons are able to quickly integrate external stimuli and translate this into gene expression changes that can be either short or persist for longer periods. This signalling network, which is important during brain development to stabilize synapses, is also involved in synaptic plasticity and thus learning, cognition and memory [87]. Recent data in mouse cortical neurons, demonstrated that activity-dependent transcriptional changes are at least partially established through rapid epigenetic alterations in a pre-programmed poised enhancer network by the early response factor FOS [74, 88••].

While these principles represent some of the emerging concepts coming from large-scale CRE identification, one of the most important insights gained from these analyses is that much of the unexplained heritability of disease phenotypes might be located in deregulated noncoding regulatory regions [12].

Misregulation of Enhancers in Disease

The importance of correct gene expression control is underscored by the misregulation or mutation of transcription factors in numerous diseases [4]. For instance, *ASCL1* mutations can give rise to Ondine's curse, a severe neurological disorder that leads to fatal sleep apnoea [89]. However, the effects of mutations within transcription factors are often pleiotropic and thus affect multiple cell types resulting in severe developmental defects that are typically incompatible with life. Instead, many disorders are characterized by more subtle tissue-specific defects. For example, mutations in the coding sequence for sonic hedgehog (*SSH*) lead to early termination of embryonic development while a mutation within an enhancer that regulates *SSH* expression in the limb bud specifically causes preaxial polydactyly [9]. While this phenotype arises from faulty expression of *SSH*, misregulation is restricted to the developing limb and therefore irrelevant in other tissues.

Early studies targeting specific genomic loci by extensive long-range mapping have established a handful of enhancers as causative in very specific disorders such as the beta-globin enhancers in Thalassemia's [90, 91] and a *RET* enhancer in Hirschsprung disease [92, 93]. After these observations, additional enhancer mutations were found in a variety of disorders including Pierre Robin syndrome [10], pancreatic agenesis [94] and congenital heart disease [95]. The latter underscored the modularity of enhancers as mutations in *TBX5* (T-box 5) result in congenital heart defects and limb malformations while mutations in single enhancers could decouple these phenotypes.

In addition, several different modes of enhancer deregulation were uncovered. In acute lymphoid leukaemia, aberrant transcriptional regulation was found to result from point mutations that created a new enhancer in front of the *TAL* (Transcription activator-like) oncogene [96]. Enhancer driven oncogene activation was also shown to occur as a result of enhancer translocations including the classic example of Burkitt lymphoma in which the *MYC* oncogene falls under the control of an immunoglobulin enhancer after a t(8;14) chromosomal translocation [97]. More recently, it was demonstrated that oncogene activation can occur after disruption of insulated chromatin neighbourhoods [98, 99]. As a result, expression of proto-oncogenes was increased through newly established long-range interactions. Finally, the epigenomic deregulation of enhancers by loss of DNA methylation was shown to be widespread in tumours [100]. These results demonstrate that diverse modes of enhancer misregulation can underlie a host of diseases and can explain the tissue-specific manifestation of such disorders.

Enhancers as a Source for Common Variation in Disease Susceptibility

With more than 400,000 potential noncoding regulatory elements identified in the human genome, the mutational space for disease-causing events has increased substantially. The inability to explain disease heritability by gene mutations alone as well as the presence of more than 85 % of disease-associated variants in noncoding DNA [101] have strengthened the notion that much of the genetic variation that is relevant to disease lies within regulatory DNA (Fig. 1b). This has been supported by earlier extensive investigation of the *RET* gene locus for which a common variant within a *RET* enhancer was found to increase Hirschsprung disease susceptibility [102, 103]. Nevertheless, linking the disease-associated variant to specific regulatory elements often remained challenging. For instance, in depth analysis of a risk haplotype in the 5' region of *SORL1* (sortilin-related receptor L) provided important new insight into the pathogenesis of Alzheimer's disease while the exact polymorphism behind this effect remained elusive [104].

Based on these observations, the integration of genome-wide association studies with datasets of annotated enhancer elements has rapidly led to the discovery of potential disease-associated variants in predicted enhancer elements (Fig. 1b) [105–110]. This revealed that common disease variants preferentially occurred at enhancers in cell types known to be affected by the disease and therefore yielded a trove of candidates for further study. For example, while common variants that alter susceptibility to behavioural disorders preferentially occurred in foetal brain CREs [106], common variants associated with increased Parkinson's disease (PD) susceptibility were found preferentially in CREs of the adult human brain (Fig. 1b) [111•]. In agreement with this, a number of neural transcription factor binding sites (e.g. Pax6 and Otx1) were disproportionately affected by variants associated with neuropsychiatric diseases and traits [106]. Several surprising observations were also made such as an unexpected link between B-cells and multiple sclerosis [106]. This suggested that specific cell types that are affected in disease could be deduced from the integration of cell type-specific enhancers and genome-wide association (GWAS) data (Fig. 1b). Similarly, in a more recent study, common variation in Alzheimer's disease was linked to the immune system suggesting that much of the regulatory variation underlying this disease may not be intrinsic to neurons [112]. This underscores the relevance of integrating the two data types to uncover new cell types that are involved in disease susceptibility but also to prioritize the regulatory elements that are likely affected by genomic variation. Furthermore, it solidifies the notion that intra-individual genetic variation, which is most pronounced at regulatory DNA [113, 114], plays an integral role in determining disease susceptibility [108–110].

Parkinson's Disease-Associated Variation Within Human Brain Enhancers

While genomic variation within CREs is likely to have functional consequences, the link between potentially relevant enhancer variants and disease has to be experimentally verified. This has been done for a handful of disorders such as the *FTO* (fat mass and obesity-associated) locus in obesity [11], *LMO1* (LIM domain only 1) in neuroblastoma predisposition [115] and *BCL11A* (B-cell lymphoma/leukaemia 11A) in sickle cell anaemia [116•]. However, proper validation is difficult, especially for complex diseases such as neurodegenerative and neuropsychiatric disorders in which the combined activity of several regulatory elements on multiple genes may underlie pathophysiology. Furthermore, given the moderate effect size of common variations on disease susceptibility, the effect size on gene expression may also be modest.

We have previously linked several genetic variants associated with altered PD susceptibility to enhancers of the adult brain [111•]. These included CREs in important *PARK* loci

such as the PARK16, PARK17 and the PARK8 loci, containing *LRKK2* (Leucine-rich repeat kinase 2), a gene that is mutated in autosomal dominant PD [117]. Similarly, we identified an intronic enhancer in the *SNCA* (α -synuclein) gene that contained two genomic variants rs356168 and rs3756054 [111•] that were in perfect linkage with earlier described PD risk alleles rs2736990 and rs11931074. These were initially discovered in cohorts of European and of Asian descent, respectively [118, 119]. This suggested that the different common variants in two populations converged at one enhancer element. In support of a role for enhancer variation, we found that both of these linked variants altered transcription factor binding sites in the predicted enhancer. Furthermore, the regulatory region acted as an enhancer in transgenic mouse assays, phenocopying the expression of *SNCA* in E11 mouse embryos. Finally, the enhancer was shown to directly target the *SNCA* promoter in chromosome conformation capture experiments in human brain tissue [111•]. These data firmly established the newly identified CRE as a bona fide *SNCA* enhancer.

As enhancers are mostly cell type- and context-specific, a correct model system and environment needs to be established to explore the functional consequences of enhancer alteration. This can be particularly difficult for the nervous system [120]. In addition, the effect size of enhancer variation is likely modest, given the fact that a 50 % increase in *SNCA* expression will cause PD [121]. A recent study dealt with all of these issues by employing an elegant experimental set up to analyse the influence of genetic variation within the intronic *SNCA* enhancer using allele-specific *SNCA* expression analysis in embryonic stem cell-derived neurons [122••]. Slight, but consistent increased expression of *SNCA* was observed for the Parkinson's variant of rs356168 but not for variant rs3756054. This suggests that the latter SNP may not contribute to disease susceptibility and that other variants with lower linkage may still have to be explored. Nevertheless, these data did confirm our previously proposed link between the rs356168 enhancer variant and PD susceptibility. This underscores the importance of rigorous validation of enhancer variants within CREs and stands as a testimony for the huge task ahead.

Conclusion

CREs play a pivotal role in the proper establishment of the gene expression programs that determine cell state. Following a decade of epigenomic exploration to chart CREs in the human genome, we are now starting to unravel some of the regulatory networks that contribute to a host of brain disorders as well as to individual variation in disease susceptibility. Since the start of the ENCODE

project, to the more recent report of 111 epigenomes by the Roadmap Epigenetics Consortium, both consortia have added hundreds of datasets of different epigenetic footprints in a host of human tissues including 8 adult and 2 foetal brain samples [6]. However, to capture the full complexity of the brain many more anatomical regions still need to be explored. Our analysis of 87 anatomically distinct regions in the human brain was a confirmation of this, as many specialized structures within the brain, that were not included in other analyses, contributed significantly to the total repertoire of predicted CREs in the human brain [111•]. Follow-up analyses to identify regulatory changes that could be relevant to brain diseases are underway with consortia such as PsychENCODE focussing on neuropsychiatric diseases including autism and schizophrenia [123]. These studies will have to be balanced between the number of epigenetic footprints analysed, the number of patients included and the number of anatomical regions required. Furthermore, new (single cell) methods will have to be optimized to tackle the investigation of rare cell types within brain tissue samples.

While many potential links between neurodegenerative disease and regulatory changes have already been revealed, extensive validation experiments are required to confirm these in detail. This is challenging because of the modest effect size of sequence variation on enhancer function and the potential involvement of multiple genes as well as the requirement of relevant model systems in which activity can be properly measured. CRISPR-Cas9 mediated engineering of human embryonic stem cells will prove a powerful tool to analyse the effect of enhancers on their cognate target gene [124], especially since these cells can be used to generate a host of different cell types in the brain [120]. However, as multiple cell types can be involved in complex diseases, the implementation of more complex culture systems such as organoid cultures, that mimic cortical development, may be of use [125, 126]. Finally, the current focus on common variation will have to be complemented with research on structural and rare variants, as those are also likely to affect enhancer activity [105]. Since transcription factor binding sites are often degenerate, allowing multiple variations to activate or inactivate enhancer elements, disease-causing mutations will likely be rare. Therefore, genetic as well as epigenetic screens to reveal rare variants and to assay their consequences on enhancer activity will need to be explored.

Compliance with Ethical Standards

Conflict of Interest Marit W. Vermunt and Menno P. Creyghton declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
 - Of major importance
1. Zhu J, Adli M, Zou JY, Verstappen G, Coyne M, Zhang X, et al. Genome-wide chromatin state transitions associated with developmental and environmental cues. *Cell*. 2013;152:642–54.
 2. Nord AS, Blow MJ, Attanasio C, Akiyama JA, Holt A, Hosseini R, et al. Rapid and pervasive changes in genome-wide enhancer usage during mammalian development. *Cell*. 2013;155:1521–31.
 3. Bulger M, Groudine M. Functional and mechanistic diversity of distal transcription enhancers. *Cell*. 2011;144:327–39.
 4. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. *Cell*. 2013;152:1237–51.
 5. Vaquerizas JM, Kummerfeld SK, Teichmann SA, Luscombe NM. A census of human transcription factors: function, expression and evolution. *Nat Rev Genet*. 2009;10:252–63.
 6. Roadmap Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, et al. Integrative analysis of 111 reference human epigenomes. *Nature*. 2015;518:317–30.
 7. Young RA. Control of the embryonic stem cell state. *Cell*. 2011;144:940–54.
 8. Takahashi K, Yamanaka S. A developmental framework for induced pluripotency. *Development*. 2015;142:3274–85.
 9. Lettice LA. A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum Mol Genet*. 2003;12:1725–35.
 10. Benko S, Fantes JA, Amiel J, Kleinjan D-J, Thomas S, Ramsay J, et al. Highly conserved non-coding elements on either side of SOX9 associated with Pierre Robin sequence. *Nat Genet*. 2009;41:359–64.
 11. Smemo S, Tena JJ, Kim K-H, Gamazon ER, Sakabe NJ, Gómez-Marín C, et al. Obesity-associated variants within FTO form long-range functional connections with IRX3. *Nature*. 2014;507:371–5.
 12. Ward LD, Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*. 2016;44:D877–81.
 13. Mitsui J, Tsuji S. Genomic aspects of sporadic neurodegenerative diseases. *Biochem Biophys Res Commun*. 2014;452:221–5.
 14. Silbereis JC, Pochareddy S, Zhu Y, Li M, Šestan N. The cellular and molecular landscapes of the developing human central nervous system. *Neuron*. 2016;89:248–68.
 15. Petryniak MA, Potter GB, Rowitch DH, Rubenstein JLR. Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron*. 2007;55:417–33.
 16. Silbereis JC, Nobuta H, Tsai H-H, Heine VM, McKinsey GL, Meijer DH, et al. Olig1 function is required to repress dlx1/2 and interneuron production in mammalian brain. *Neuron*. 2014;81:574–87.
 17. Sansom SN, Griffiths DS, Faedo A, Kleinjan D-J, Ruan Y, Smith J, et al. The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis. *PLoS Genet*. 2009;5:e1000511.
 18. Heins N, Malatesta P, Cecconi F, Nakafuku M, Tucker KL, Hack MA, et al. Glial cells generate neurons: the role of the transcription factor Pax6. *Nat Neurosci*. 2002;5:308–15.
 19. Berninger B, Costa MR, Koch U, Schroeder T, Sutor B, Grothe B, et al. Functional properties of neurons derived from in vitro reprogrammed postnatal astroglia. *J Neurosci Soc Neurosci*. 2007;27:8654–64.
 20. Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Südhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010;463:1035–41.
 21. Ang CE, Wernig M. Induced neuronal reprogramming. *J Comp Neurol*. 2014;522:2877–86.
 22. Miller JA, Ding S-L, Sunkin SM, Smith KA, Ng L, Szafer A, et al. Transcriptional landscape of the prenatal human brain. *Nature*. 2014;508:199–206.
 23. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature*. 2012;489:391–9.
 24. Konopka G, Friedrich T, Davis-Turak J, Winden K, Oldham MC, Gao F, et al. Human-specific transcriptional networks in the brain. *Neuron*. 2012;75:601–17.
 25. Kang HJ, Kawasawa YI, Cheng F, Zhu Y, Xu X, Li M, et al. Spatio-temporal transcriptome of the human brain. *Nature*. 2011;478:483–9.
 26. Bayatti N, Sarma S, Shaw C, Eyre JA, Vouyiouklis DA, Lindsay S, et al. Progressive loss of PAX6, TBR2, NEUROD and TBR1 mRNA gradients correlates with translocation of EMX2 to the cortical plate during human cortical development. *Eur J Neurosci*. 2008;28:1449–56.
 27. Hardison RC, Taylor J. Genomic approaches towards finding cis-regulatory modules in animals. *Nat Rev Genet*. 2012;13:469–83.
 28. Jolma A, Yan J, Whittington T, Toivonen J, Nitta KR, Rastas P, et al. DNA-binding specificities of human transcription factors. *Cell*. 2013;152:327–39.
 29. Rubinstein M, de Souza FSJ. Evolution of transcriptional enhancers and animal diversity. *Philos Trans R Soc Lond B Biol Sci*. 2013;368:20130017–7.
 30. Biggin MD. Animal transcription networks as highly connected, quantitative continua. *Dev Cell*. 2011;21:611–26.
 31. Stampfel G, Kazmar T, Frank O, Wienerroither S, Reiter F, Stark A. Transcriptional regulators form diverse groups with context-dependent regulatory functions. *Nature*. 2015;528:147–51.
 32. Spitz F, Furlong EEM. Transcription factors: from enhancer binding to developmental control. *Nat Rev Genet*. 2012;13:613–26.
 33. Li X-Y, MacArthur S, Bourgon R, Nix D, Pollard DA, Iyer VN, et al. Transcription factors bind thousands of active and inactive regions in the Drosophila blastoderm. *PLoS Biol*. 2008;6:e27.
 34. Smale ST. Pioneer factors in embryonic stem cells and differentiation. *Curr Opin Genet Dev*. 2010;20:519–26.
 35. Smale ST. Transcriptional regulation in the innate immune system. *Curr Opin Immunol*. 2012;24:51–7.
 36. Calo E, Wysocka J. Modification of enhancer chromatin: what, how, and why? *Mol Cell*. 2013;49:825–37.
 37. de Laat W, Duboule D. Topology of mammalian developmental enhancers and their regulatory landscapes. *Nature*. 2013;502:499–506.
 38. Dekker J, Heard E. Structural and functional diversity of topologically associating domains. *FEBS Lett*. 2015;589:2877–84.
 39. Kagey MH, Newman JJ, Bilodeau S, Zhan Y, Orlando DA, van Berkum NL, et al. Mediator and cohesin connect gene expression and chromatin architecture. *Nature*. 2010;467:430–5.
 40. Seitan VC, Faure AJ, Zhan Y, McCord RP, Lajoie BR, Ing-Simmons E, et al. Cohesin-based chromatin interactions enable regulated gene expression within preexisting architectural compartments. *Genome Res*. 2013;23:2066–77.

41. Phillips-Cremins JE, Corces VG. Chromatin insulators: linking genome organization to cellular function. *Mol Cell*. 2013;50:461–74.
42. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature*. 2012;485:376–80.
43. Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature*. 2012;485:381–5.
44. de Souza FSJ, Santangelo AM, Bumashny V, Avale ME, Smart JL, Low MJ, et al. Identification of neuronal enhancers of the proopiomelanocortin gene by transgenic mouse analysis and phylogenetic footprinting. *Mol Cell Biol*. 2005;25:3076–86.
45. Banerji J, Rusconi S, Schaffner W. Expression of a beta-globin gene is enhanced by remote SV40 DNA sequences. *Cell*. 1981;27:299–308.
46. Trudel M, Costantini F. A 3' enhancer contributes to the stage-specific expression of the human beta-globin gene. *Genes Dev*. 1987;1:954–61.
47. Kleinjan DA, Seawright A, Mella S, Carr CB, Tyas DA, Simpson TI, et al. Long-range downstream enhancers are essential for Pax6 expression. *Dev Biol*. 2006;299:563–81.
48. Nobrega MA, Ovcharenko I, Afzal V, Rubin EM. Scanning human gene deserts for long-range enhancers. *Science*. 2003;302:413–3.
49. Pennacchio LA, Ahituv N, Moses AM, Prabhakar S, Nobrega MA, Shoukry M, et al. In vivo enhancer analysis of human conserved non-coding sequences. *Nature*. 2006;444:499–502.
50. Visel A, Prabhakar S, Akiyama JA, Shoukry M, Lewis KD, Holt A, et al. Ultraconservation identifies a small subset of extremely constrained developmental enhancers. *Nat Genet*. 2008;40:158–60.
51. Hare EE, Peterson BK, Iyer VN, Meier R, Eisen MB. Sepsid even-skipped enhancers are functionally conserved in *Drosophila* despite lack of sequence conservation. *PLoS Genet*. 2008;4:e1000106.
52. Fisher S, Grice EA, Vinton RM, Bessling SL, McCallion AS. Conservation of RET regulatory function from human to zebrafish without sequence similarity. *Science*. 2006;312:276–9.
53. Mikkelsen TS, Ku M, Jaffe DB, Issac B, Lieberman E, Giannoukos G, et al. Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature*. 2007;448:553–60.
54. Heintzman ND, Hon GC, Hawkins RD, Kheradpour P, Stark A, Harp LF, et al. Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature*. 2009;459:108–12.
55. John S, Sabo PJ, Canfield TK, Lee K, Vong S, Weaver M, et al. Genome-scale mapping of DNase I hypersensitivity. *Curr Protoc Mol Biol*. 2013;27(21):27.
56. Giresi PG, Kim J, McDaniell RM, Iyer VR, Lieb JD. FAIRE (Formaldehyde-Assisted Isolation of Regulatory Elements) isolates active regulatory elements from human chromatin. *Genome Res*. 2007;17:877–85.
57. Buenrostro JD, Wu B, Chang HY, Greenleaf WJ. ATAC-seq: a method for assaying chromatin accessibility genome-wide. *Curr Protoc Mol Biol*. 2015;109:21.29.1–9.
58. Phillips-Cremins JE, Sauria MEG, Sanyal A, Gerasimova TI, Lajoie BR, Bell JSK, et al. Architectural protein subclasses shape 3D organization of genomes during lineage commitment. *Cell*. 2013;153:1281–95.
59. Chong JA, Tapia-Ramírez J, Kim S, Toledo-Aral JJ, Zheng Y, Boutros MC, et al. REST: a mammalian silencer protein that restricts sodium channel gene expression to neurons. *Cell*. 1995;80:949–57.
60. Gross DS, Garrard WT. Nuclease hypersensitive sites in chromatin. *Annu Rev Biochem*. 1988;57:159–97.
61. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nat Genet*. 2007;39:311–8.
62. Lan F, Collins RE, De Cegli R, Alpatov R, Horton JR, Shi X, et al. Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. *Nature*. 2007;448:718–22.
63. Ooi SKT, Qiu C, Bernstein E, Li K, Jia D, Yang Z, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. *Nature*. 2007;448:714–7.
64. Jeong KW, Kim K, Situ AJ, Ulmer TS, An W, Stallcup MR. Recognition of enhancer element-specific histone methylation by TIP60 in transcriptional activation. *Nat Struct Mol Biol*. 2011;18:1358–65.
65. Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J. A unique chromatin signature uncovers early developmental enhancers in humans. *Nature*. 2011;470:279–83.
66. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*. 2006;125:315–26.
67. Creighton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U S A*. 2010;107:21931–6.
68. Bonn S, Zinzen RP, Girardot C, Gustafson EH, Perez-Gonzalez A, Delhomme N, et al. Tissue-specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development. *Nat Genet*. 2012;44:148–56.
69. Wang Z, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, et al. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet*. 2008;40:897–903.
70. Visel A, Blow MJ, Li Z, Zhang T, Akiyama JA, Holt A, et al. ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature*. 2009;457:854–8.
71. Filippakopoulos P, Picaud S, Mangos M, Keates T, Lambert J-P, Barsyte-Lovejoy D, et al. Histone recognition and large-scale structural analysis of the human bromodomain family. *Cell*. 2012;149:214–31.
72. Fenley AT, Adams DA, Onufriev AV. Charge state of the globular histone core controls stability of the nucleosome. *Biophys J*. 2010;99:1577–85.
73. Arnold CD, Gerlach D, Stelzer C, Boryń LM, Rath M, Stark A. Genome-wide quantitative enhancer activity maps identified by STARR-seq. *Science*. 2013;339:1074–7.
74. Kim T-K, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, et al. Widespread transcription at neuronal activity-regulated enhancers. *Nature*. 2010;465:182–7.
75. Andersson R, Gebhard C, Miguel-Escalada I, Hoof I, Bornholdt J, Boyd M, et al. An atlas of active enhancers across human cell types and tissues. *Nature*. 2014;507:455–61.
76. de Wit E, Bouwman BAM, Zhu Y, Klous P, Splinter E, Verstegen MJAM, et al. The pluripotent genome in three dimensions is shaped around pluripotency factors. *Nature*. 2013;501:227–31.
77. Odom DT, Dowell RD, Jacobsen ES, Gordon W, Danford TW, MacIsaac KD, et al. Tissue-specific transcriptional regulation has diverged significantly between human and mouse. *Nat Genet*. 2007;39:730–2.
78. Villar D, Berthelot C, Aldridge S, Rayner TF, Lukk M, Pignatelli M, et al. Enhancer evolution across 20 mammalian species. *Cell*. 2015;160:554–66.
79. Vermunt MW, Tan SC, Castelijns B, Geeven G, Reinink P, de Bruijn E, et al. Epigenomic annotation of gene regulatory alterations during evolution of the primate brain. *Nat Neurosci*. 2016;19:494–503.

80. Reilly SK, Yin J, Ayoub AE, Emera D, Leng J, Cotney J, et al. Evolutionary genomics. Evolutionary changes in promoter and enhancer activity during human corticogenesis. *Science*. 2015;347:1155–9.
81. Vietri Rudan M, Barrington C, Henderson S, Ernst C, Odom DT, Tanay A, et al. Comparative Hi-C reveals that CTCF underlies evolution of chromosomal domain architecture. *Cell Rep*. 2015;10:1297–309.
82. Hong J-W, Hendrix DA, Levine MS. Shadow enhancers as a source of evolutionary novelty. *Science*. 2008;321:1314–4.
83. Perry MW, Boettiger AN, Bothma JP, Levine M. Shadow enhancers foster robustness of *Drosophila* gastrulation. *Curr Biol*. 2010;20:1562–7.
84. Swanson CI, Evans NC, Barolo S. Structural rules and complex regulatory circuitry constrain expression of a Notch- and EGFR-regulated eye enhancer. *Dev Cell*. 2010;18:359–70.
85. McGaughey DM, Vinton RM, Huynh J, Al-Saif A, Beer MA, McCallion AS. Metrics of sequence constraint overlook regulatory sequences in an exhaustive analysis at *phox2b*. *Genome Res*. 2008;18:252–60.
86. Domené S, Bumashny VF, De Souza FSJ, Franchini LF, Nasif S, Low MJ, et al. Enhancer turnover and conserved regulatory function in vertebrate evolution. *Philos Trans R Soc Lond B Biol Sci*. 2013;368:20130027–7.
87. West AE, Greenberg ME. Neuronal activity-regulated gene transcription in synapse development and cognitive function. *Cold Spring Harb Perspect Biol*. 2011;3:a005744–4.
88. Malik AN, Vierbuchen T, Hemberg M, Rubin AA, Ling E, Couch CH, et al. Genome-wide identification and characterization of functional neuronal activity-dependent enhancers. *Nat Neurosci*. 2014;17:1330–9. **This work demonstrates rapid activation of a predetermined enhancer network upon membrane depolarization in neurons. It represents a first step towards understanding how enhancer networks control gene expression changes during learning and memory formation in the brain.**
89. de Pontual L, Népote V, Attié-Bitach T, Halabiah Al H, Trang H, Elghouzzi V, et al. Noradrenergic neuronal development is impaired by mutation of the proneural *HASH-1* gene in congenital central hypoventilation syndrome (Ondine's curse). *Hum Mol Genet*. 2003;12:3173–80.
90. Kioussis D, Vanin E, deLange T, Flavell RA, Grosveld FG. Beta-globin gene inactivation by DNA translocation in gamma beta-thalassaemia. *Nature*. 1983;306:662–6.
91. Semenza GL, Delgrosso K, Poncz M, Malladi P, Schwartz E, Surrey S. The silent carrier allele: beta thalassemia without a mutation in the beta-globin gene or its immediate flanking regions. *Cell*. 1984;39:123–8.
92. Bolk S, Pelet A, Hofstra RM, Angrist M, Salomon R, Croaker D, et al. A human model for multigenic inheritance: phenotypic expression in Hirschsprung disease requires both the *RET* gene and a new 9q31 locus. *Proc Natl Acad Sci*. 2000;97:268–73.
93. Gabriel SB, Salomon R, Pelet A, Angrist M, Amiel J, Fornage M, et al. Segregation at three loci explains familial and population risk in Hirschsprung disease. *Nat Genet*. 2002;31:89–93.
94. Weedon MN, Cebola I, Patch A-M, Flanagan SE, De Franco E, Caswell R, et al. Recessive mutations in a distal *PTF1A* enhancer cause isolated pancreatic agenesis. *Nat Genet*. 2014;46:61–4.
95. Smemo S, Campos LC, Moskowitz IP, Krieger JE, Pereira AC, Nobrega MA. Regulatory variation in a *TBX5* enhancer leads to isolated congenital heart disease. *Hum Mol Genet*. 2012;21:3255–63.
96. Mansour MR, Abraham BJ, Anders L, Berezovskaya A, Gutierrez A, Durbin AD, et al. Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. *Science*. 2014;346:1373–7.
97. Wittekindt NE, Hörtnagel K, Geltinger C, Polack A. Activation of c-myc promoter P1 by immunoglobulin kappa gene enhancers in Burkitt lymphoma: functional characterization of the intron enhancer motifs kappaB, E box 1 and E box 2, and of the 3' enhancer motif PU. *Nucleic Acids Res*. 2000;28:800–8.
98. Hnisz D, Weintraub AS, Day DS, Valton A-L, Bak RO, Li CH, et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. *Science*. 2016;351:1454–8.
99. Flavahan WA, Drier Y, Liau BB, Gillespie SM, Venteicher AS, Stemmer-Rachamimov AO, et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. *Nature*. 2016;529:110–4.
100. Aran D, Hellman A. DNA methylation of transcriptional enhancers and cancer predisposition. *Cell*. 2013;154:11–3.
101. Freedman ML, Monteiro ANA, Gayther SA, Coetzee GA, Risch A, Plass C, et al. Principles for the post-GWAS functional characterization of cancer risk loci. *Nat Genet*. 2011;43:513–8.
102. Emison ES, McCallion AS, Kashuk CS, Bush RT, Grice E, Lin S, et al. A common sex-dependent mutation in a *RET* enhancer underlies Hirschsprung disease risk. *Nature*. 2005;434:857–63.
103. Grice EA, Rochelle ES, Green ED, Chakravarti A, McCallion AS. Evaluation of the *RET* regulatory landscape reveals the biological relevance of a HSCR-implicated enhancer. *Hum Mol Genet*. 2005;14:3837–45.
104. Young JE, Boulanger-Weill J, Williams DA, Woodruff G, Buen F, Revilla AC, et al. Elucidating molecular phenotypes caused by the *SORL1* Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. *Cell Stem Cell*. 2015;16:373–85.
105. Khurana E, Fu Y, Colonna V, Mu XJ, Kang HM, Lappalainen T, et al. Integrative annotation of variants from 1092 humans: application to cancer genomics. *Science*. 2013;342:1235587–7.
106. Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, et al. Systematic localization of common disease-associated variation in regulatory DNA. *Science*. 2012;337:1190–5.
107. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature*. 2012;489:57–74.
108. Kasowski M, Kyriazopoulou-Panagiotopoulou S, Grubert F, Zaugg JB, Kundaje A, Liu Y, et al. Extensive variation in chromatin states across humans. *Science*. 2013;342:750–2.
109. Kilpinen H, Waszak SM, Gschwind AR, Raghav SK, Witwicki RM, Orioli A, et al. Coordinated effects of sequence variation on DNA binding, chromatin structure, and transcription. *Science*. 2013;342:744–7.
110. McVicker G, van de Geijn B, Degner JF, Cain CE, Banovich NE, Raj A, et al. Identification of genetic variants that affect histone modifications in human cells. *Science*. 2013;342:747–9.
111. Vermunt MW, Reinink P, Korving J, de Bruijn E, Creyghton PM, Basak O, et al. Large-scale identification of coregulated enhancer networks in the adult human brain. *Cell Rep*. 2014;9:767–79. **In this work enhancers are identified across anatomical regions of the human brain and used to prioritize common variants linked to neurodegenerative disease.**
112. Gjoneska E, Pfenning AR, Mathys H, Quon G, Kundaje A, Tsai L-H, et al. Conserved epigenomic signals in mice and humans reveal immune basis of Alzheimer's disease. *Nature*. 2015;518:365–9.
113. Schaub MA, Boyle AP, Kundaje A, Batzoglou S, Snyder M. Linking disease associations with regulatory information in the human genome. *Genome Res*. 2012;22:1748–59.
114. Ward LD, Kellis M. Interpreting noncoding genetic variation in complex traits and human disease. *Nat Biotechnol*. 2012;30:1095–106.
115. Oldridge DA, Wood AC, Weichert-Leahey N, Crimmins I, Sussman R, Winter C, et al. Genetic predisposition to neuroblastoma mediated by a *LMO1* super-enhancer polymorphism. *Nature*. 2015;528:418–21.

116. • Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science*. 2013;342:253–7. **This work is an excellent example of how cell type-specific enhancer regulation is affected by common variation. This is shown to have physiological consequences that can be relevant for therapeutic strategies in disease.**
117. Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. *Neuron*. 2004;44:601–7.
118. Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. *Nat Genet*. 2009;41:1308–12.
119. Satake W, Nakabayashi Y, Mizuta I, Hirota Y, Ito C, Kubo M, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet*. 2009;41:1303–7.
120. Brennand KJ, Marchetto MC, Benvenisty N, Brüstle O, Ebert A, Izpisua Belmonte JC, et al. Creating patient-specific neural cells for the in vitro study of brain disorders. *Stem Cell Rep*. 2015;5: 933–45.
121. Devine MJ, Gwinn K, Singleton A, Hardy J. Parkinson's disease and α -synuclein expression. *Mov Disord*. 2011;26:2160–8.
122. • Soldner F, Stelzer Y, Shivalila CS, Abraham BJ, Latourelle JC, Barrasa MI, et al. Parkinson-associated risk variant in distal enhancer of α -synuclein modulates target gene expression. *Nature*. 2016;533:95–9. **In this work the authors demonstrate how common variation in enhancers can have extremely small but yet significant effects on target gene expression. The work highlights the importance of the need for rigorous testing of enhancer variation in a well-defined environment.**
123. Akbarian S, Liu C, Knowles JA, Vaccarino FM, Farnham PJ, Crawford GE, et al. The PsychENCODE project. *Nat Neurosci*. 2015;18:1707–12.
124. Hockemeyer D, Jaenisch R. Induced pluripotent stem cells meet genome editing. *Cell Stem Cell*. 2016;18:573–86.
125. Lancaster MA, Renner M, Martin C-A, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501:373–9.
126. Eiraku M, Watanabe K, Matsuo-Takasaki M, Kawada M, Yonemura S, Matsumura M, et al. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. *Cell Stem Cell*. 2008;3:519–32.