Characterising the genetic basis of immune response variation to identify causal mechanisms underlying disease susceptibility

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6 **Characterising the genetic basis of immune response variation to**
7 **identify causal mechanisms underlying disease susceptibility**
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9 Short title: genetic basis of immune response variation
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38 genetics, selective constraint, adaptation.

Abstract (250 words)

Over the last ten years, genome-wide association studies (GWAS) have identified hundreds of susceptibility loci for autoimmune diseases. However, despite increasing power for the detection of both common and rare coding variants affecting disease susceptibility, a large fraction of disease heritability has remained unexplained. In addition, a majority of the identified loci are located in non-coding regions, and translation of disease-associated loci into new biological insights on the aetiology of immune disorders has been lagging. This highlights the need for a better understanding of non-coding variation and new strategies to identify causal genes at disease loci. In this review, I will first detail the molecular basis of gene expression and review the various mechanisms that contribute to alter gene activity at the transcriptional and post-transcriptional level. I will then review the findings from 10 years of functional genomics studies regarding the genetics on gene expression, in particular in the context of infection. Finally, I will discuss the extent to which genetic variants that modulate gene expression at transcriptional and post-transcriptional level contribute to disease susceptibility and present strategies to leverage these information for the identification of causal mechanisms at disease loci in the era of whole genome sequencing.

59 ***Introduction***

60 The host immune response to stress exhibits considerable variation, both at the
61 individual and population levels¹. While many non heritable factors contribute to this
62 variability, including age, gender or past exposures to pathogens²⁻⁵ (see¹ for a review
63 on these aspects). There is also widespread evidence that genetics plays a
64 significant role in determining the efficacy of the human immune response, in
65 particular in the context of innate immunity⁶⁻¹². Genome-wide association studies
66 (GWAS) have been a considerable asset in defining the genomic regions involved in
67 immune disease susceptibility¹³. However, identifying the causal mechanisms
68 through which GWAS variants contribute to disease susceptibility has proven to be
69 extremely challenging. Indeed, it was soon realized that most GWAS pointed toward
70 gene deserts or non-coding regions^{14,15}, highlighting the strong need for
71 characterization of the molecular mechanisms through which such non-coding
72 regions may alter immune disease susceptibility¹⁶. Furthermore, while large GWAS
73 meta-analyses have granted increasing power to capture the effect of common
74 variants on disease susceptibility, a large fraction of disease heritability has
75 remained unaccounted for¹⁷. There is now increasing evidence to suggest that rare
76 variants, non-coding in particular, may contribute to explain this missing heritability
77 ¹⁸⁻²⁰. Therefore, there is a growing need to understand how non-coding variants,

whether rare or common, contribute to the variability of immune phenotypes in humans. In this review, I will first present the various mechanisms through which non-coding elements may contribute to regulate gene expression, either at the transcriptional level, or post-transcriptionally through RNA splicing and degradation. Then, I will review current knowledge of how common genetic variants drive immune gene expression at the transcriptional and post-transcriptional level. Finally, I will discuss strategies to decipher the impact of rare regulatory variants on immune response and characterize the causal mechanisms that determine immune disease susceptibility.

The molecular bases of gene expression

In multi-cellular organisms, tight regulation of gene expression programs is essential. First, during development, cells must coordinate expression of various transcription factors to divide and differentiate into future organs. Then, in adult life, regulatory sequence need to allow for cell-type specific gene expression profiles, that are both (i) robust to genetic variations and (ii) able to react appropriately to external stimuli. In human, such combination of resilience and adaptability is achieved through a network of regulatory features, the complexity of which largely exceeds that of the protein coding genome. Indeed, while coding sequence represent only 1.5% of the

human genome, there is evidence that at least 20% of the genome is covered by regulatory features, and 10% is actively bound by known transcription factors²¹.

Promoters and enhancers shape transcriptional activity across tissues

The regulatory features that control transcription are characterized by epigenetic histone modifications (see **Table 1** and ref.²²) and can be decomposed in two main categories (**Figure 1a**). At the transcription start site, promoters recruit RNA polymerase, along with a variety of other transcription factors, to initiate gene transcription^{15,23,24}. While 96% of promoters are active in more than one tissue or cell type, only ~20% are ubiquitously expressed indicative of strong tissue-specificity of gene regulation²³. Tissue-specificity is further increased by distal enhancer regions — located up to 1Mb away from the promoters they regulate — which, when activated by tissue-specific transcription factors, are brought in contact with their associated promoter by looping of the DNA molecule (**Figure 1b,c**) to modulate their activity^{15,24,25}. Activation of both promoters and enhancers is associated to an opening of the chromatin and acetylation of H3K27 histone residues (**Figure 1d,e**), together with transcription of non-coding RNAs^{22,25,26}. This transcription occurs either in the opposite direction of transcriptional elongation at promoters (upstream antisense RNAs), or bi-directionally for enhancers (enhancer RNAs). Although such

116 RNAs are rapidly degraded by exosomes, they allow precise quantification of
117 enhancer/promoter activity by sequencing techniques that target the 5' end of
118 transcripts such as CAGE-seq or GRO-Cap^{23,25,27}.

119 Over the last 10 years, large Consortia — such as Fantom5^{23,25}, ENCODE²⁶,
120 Blueprint²⁸⁻³¹ or Roadmap Epigenomic²² — have combined these techniques with
121 Chromatin immuno-precipitation (ChIPSeq) and chromatin accessibility Assays
122 (DNase-Seq, FAIRE-Seq, or ATAC-Seq) to precisely map promoters and enhancers
123 along the genome and characterize of their activity across tissues. In doing so,
124 theses studies have provided valuable insights on the regulatory architecture of the
125 response to immune stimuli. Notably, immune enhancers were shown to have a
126 distinct sequence composition, DNA structure and activity patterns³², with their
127 activation preceding that of their proximal promoters during immune response,
128 revealing their crucial role in the early transcriptional response to immune stimuli³³.

130 ***Three-dimensional chromatin conformation orchestrates transcription***

131 Because enhancers are located far from the genes they regulate,
132 understanding how they are brought into contact with their target gene is of primary
133 importance to decipher the complex networks of that underlies transcriptional
134 regulation in humans. Chromosome Conformation Capture techniques (Hi-C, and

Promoter-capture Hi-C, Chia-PET) have recently enabled the detection of DNA-DNA interactions along the genome^{30,31,34,35}, allowing the assignment of enhancers to their target genes. In practice, a single gene is often regulated by multiple enhancers, and one enhancer can regulate multiple genes^{15,24}. Notably, ~70% of these interactions are restricted to specific regions, known as Topologically associating domains or TADs, which can spread across hundreds of kilobases^{24,31}. The boundaries of these TADs are genetically encoded — with evidence of TADs being modified by targeted deletions and genetic variants occurring at the boundary^{35,36} — and are enriched in binding sites of the insulating factor CTCF. Note, however, that CTCF binding sites have also been observed within TADs³⁵, indicating that CTCF binding is not sufficient to induce a TAD boundary. Growing evidence suggests that TADs act to facilitate efficient and insulated co-regulation of functionally related genes. In particular, they were reported to segregate promoters of immune gene together to facilitate their epigenetic priming³⁷.

Within TADs, promoter/enhancer conformation exhibits a wide variability across tissues, and was proposed to underlie cell type-specificity of enhancer functions³¹. In particular, several studies have reported the existence of enhancers acting as hubs for chromatin-chromatin interactions, linking promoters to other enhancers^{30,38,39}. These hubs — sometimes referred as super-enhancers³⁸,

frequently interacting regions³⁰ or initiator enhancers³⁹ — are tissue-specific³⁰, preferentially found next to cell identity genes^{30,38} and enriched in tissue-specific eQTL³⁹. More importantly, super enhancers are strongly enriched in disease-related SNPs and largely contribute to auto-immunity^{30,38}. For instance, up to 22% of SLE GWAS loci fall into B-cell specific super enhancers, with disease-linked super-enhancers being observed at key immune genes such as HLA-DRB1, HLA-DQA1, the interferon response factor IRF5 or the NF- κ B inhibitor TNFAIP3³⁸.

Post-transcriptional regulation through alternative splicing

Beyond transcription, the control of gene expression is also ensured at the post-transcriptional level through alternative splicing (AS) and miRNA-mediated degradation. AS can change the function of the protein isoform that is being expressed, through addition/removal of exons encoding functional domains. The most iconic example of such a mechanism is the splicing transition of CD45 antigen during training of adaptive immunity, where T-cells switch from the CD45RO epitope that characterizes naïve T-cells to the CD45RA epitope associated with memory T-cells⁴⁰. AS can also repress gene expression through the introduction of premature stop codons into transcribed sequences, leading to non-sense mediated decay^{41,42}. Although our understanding of the splicing code remains incomplete, there is a wide agreement that it involves the recognition by the spliceosome of donor/acceptor

sequences located at the intron boundaries, together with a branchpoint sequence — typically located ~30 nucleotide upstream of the acceptor splice site^{41,42}. This leads to the formation of a lariat and the removal of the intron. AS is induced by competition between splice sites and is regulated through a wide array of RNA binding proteins able to recognize both exonic and intronic regulatory sequences⁴²⁻⁴⁵. These sequences can act as to either repress or promote, the recruitment of the spliceosome around a specific splice site or exon. In addition, alternative splicing can also be regulated co-transcriptionally, with exon inclusion being associated to a lower transcriptional elongation rate around the alternatively spliced exon⁴¹.

Impact of miRNA-mediated degradation on immune response

Another layer of post-transcriptional regulation occurs through the binding of miRNAs to 3'UTRs, and the targeting of bound mRNAs to the RNA induced silencing complex (RISC)^{46,47}. The RISC then functions to repress gene expression through degradation of transcripts and inhibition of translation. For example, upon TLR4 stimulation, the NF- κ B-activated miRNAs mir-146a and miR-155 are strongly up-regulated and bind to 3'UTRs of the TLR4 signal transducers, TRAF6 and IRAK4 and the TLR4 inhibitors SHIP1 and SOCS1⁴⁸, respectively. These mRNAs are then targeted to the RISC for degradation, thereby contributing to the balance of

inflammatory responses to TLR4 stimulation. Several mechanisms can further modulate the impact of miRNAs on gene expression. For instance, usage of alternative UTRs and poly-adenylation sites can change the set of miRNA binding sites available for regulation of gene expression⁴⁹. Such changes have been shown to be widespread upon stimulation⁴⁹ and to play an important role in allowing key regulators of immune response, such as IRF5 or Map Kinases MAPKAP1 and MAP2K4, to escape repression by immune miRNAs⁴⁹. Formation of miRNA isoforms — known as isomiRs — could also contribute to immune response variability. Indeed, it was shown that post-transcriptional modifications of miRNAs through editing of the miRNA seed sequence or shifts in 5'/3' boundaries of the mature miRNA, can alter their target sites or stability and lead to a decrease in their ability to regulate mRNAs^{46,50}. Furthermore, extensive changes in isomiRs have been observed upon stimulation, including for key immune miRNAs⁵¹. For instance, upon stimulation by interferon, mir-222 expresses shorter isoforms, which are associated to lower apoptosis^{52,53}, highlighting how changes of miRNA isoform can contribute to regulate the response to immune stimuli.

The genetic bases of gene expression

211 One major challenge of functional genomics is the understanding of the regulatory
212 code that dictates gene expression from the genetic information encoded by DNA
213 sequences. One approach to learn this regulatory code is through the use of
214 naturally-occurring genetic variation. Studies of expression quantitative trait loci
215 (eQTL) aim to identify genetic variants that are associated to gene expression levels
216 in human populations.

217

218 ***Genetic control of transcriptional activity is highly stimulation-specific***

219 eQTL studies have first focused of steady-state expression measurements on
220 lymphoblastoid cell lines or whole blood, from a wide range of populations⁵⁴⁻⁵⁸.
221 These studies are revealed that the majority of eQTLs are located in *cis*, i.e. less
222 than 1Mb away of the gene they control, and that population-specific eQTLs are
223 driven by changes in allelic frequency rather than gene $\times$ gene or gene $\times$
224 environment interactions⁵⁹. While a few *trans*-associations — involving genes
225 located on different chromosomes — were detected, these associations were either
226 weak or driven by cross mapping errors⁶⁰ and changes in cellular composition⁶¹. The
227 strong potential of eQTL for biomedical applications⁶² has rapidly lead to extend this
228 framework to multiple tissues⁶³ or purified immune cell types^{29,64}, revealing a strong
229 tissue specificity of eQTLs. Furthermore, exposing human cells to immune and

infectious challenges has allowed the identification of response eQTLs that are associated with changes in gene expression specifically upon immune stimulation (Figure 1f,g)^{6,9,12,65,66}. Interestingly, response-eQTLs were found to be highly variable across stimuli and time points^{8,9,12}. For instance, the rs9266257 variant was found to control expression of HLA-C in macrophages specifically upon infection by Salmonella, but not Listeria⁶⁵. Interestingly, this phenomenon appeared even more striking among trans-eQTLs which were often revealed by immune stimulation⁸. An outstanding example of this is the TLR1 locus, where the European-specific missense variant rs5743618 is associated with a decreased inflammatory response to stimulation of the TLR1/2 complex⁹. Interestingly, the European-specific allele of this variant is protective against leprosis⁶⁷ and overlaps one of the strongest signature of natural selection in the human genome⁹, highlighting how selective pressures induced by pathogens can lead to strong inter-individual and inter-population differences in immune phenotypes.

The subtle effect of enhancer variants of gene expression

Studies of the mechanistic bases of eQTLs^{9,57} have shown that promoter variants are largely overrepresented among eQTLs. However, enhancer variants are increasingly likely to be found when focusing on secondary, weaker eQTLs⁶³. The

249 increasing power to detect small effect size and highly localized effects offered by
250 eQTL meta-analyses⁶¹ and analyses of eQTLs at single cell resolution⁶⁸ will likely
251 improve our understanding of the contribution of enhancer variants to gene
252 expression. Another approach to detect subtle effects of enhancers variants on gene
253 expression consists in assaying directly the effect of genetic variants on regulatory
254 activity, based on chromatin accessibility^{66,69}, histone modifications^{29,69}, or short-live
255 transcriptional products⁷⁰. In doing so, Li *et al.* estimated that QTLs for regulatory
256 elements could account for ~60% of eQTLs, but only <25% of enhancers QTLs were
257 associated to an effect on transcription nearest gene⁶⁹, suggesting that only a small
258 fraction of variants that alter regulatory activity lead to a measurable effect on gene
259 expression. However, variants that alter chromatin accessibility with no visible effect
260 on gene expression at basal state can contribute to prime enhancers⁶⁶. Upon
261 stimulation, this priming favours the binding of stimulus-specific transcription factors,
262 such as NF- κ B or Stat2, leading to a stronger immune response^{37,66}. Together, these
263 observations highlight the potential of eQTL meta-analyses and direct assays of
264 regulatory activity to understand inter-individual variability in immune response.

265

266 ***Genetic regulation of post-transcriptional variability in the immune response***

267 Perhaps unsurprisingly given their strong potential to alter protein function,

268 splicing quantitative trait loci (sQTLs, **figure 2a**) have turned out to be consistently

269 less frequent than eQTLs across the human genome. Indeed, eQTLs appear to be

270 from 1.5 to 6 time more frequent than sQTLs, across studies^{56,57,58,65,71,72} (**Table 2**).

271 In addition, sQTLs displayed a stronger overlap between conditions of stimulation

272 than eQTLs^{65,71}, suggesting a limited impact of stimulation on the genetic regulation

273 of splicing. Nevertheless, disease-causing sQTL have been observed at many key

274 immune genes including OAS1, IRF7, IL7R, IFI44L, TYK2 and ERAP2^{65,71,73-75},

275 highlighting the importance of considering splicing variation when searching for the

276 causal mechanisms underlying GWAS loci. Interestingly, several of these loci,

277 including *OAS1* or *ERAP2* present haplotypic signatures of positive and balancing

278 selection, suggesting that these sQTL have conferred a selective advantage to

279 human populations in the past, likely through increased resistance to pathogens.^{71,73}.

280 The regulation of miRNA expression has also been extensively studied, both at basal

281 state^{76,77} and in response to infection⁷⁸. Similar to splicing, miRNA QTLs studies

282 (**figure 2b**) have generally suggested a limited genetic control of miRNAs, consistent

283 with expectation of strong selective constraints on miRNA expression due to their

widespread impact on gene expression^{76,78}. miRNA QTLs appear to be strongly enriched in variants located in their promoter and eQTLs of their host gene⁷⁹. However, there is also strong evidence that enhancers and loci that are bound by transcription factor (TF) contribute to miRNA expression⁷⁷. Recently, a study has used models of SNP-miRNA interactions to quantify the impact of SNPs on the regulation by miRNAs (reg-eQTL, **figure 2c**). This has lead to the identification of ~40 SNP that disrupt miRNA binding sites⁸⁰. The proposed approach, however, suffers from the lack of power usually associated with interaction testing, and may largely underestimate the true number of such reg-eQTL. Larger studies combining expression, genetic and miRNA data are now needed to fully characterize the impact of genetic variants on miRNA-mediated degradation in the immune response.

The contribution of regulatory variants to disease susceptibility

In support of the importance of eQTLs in disease susceptibility, they have been repeatedly reported to be enriched in disease loci^{8,9,12,57,63}. For instance, the association of the rs4077515 variant with Inflammatory bowel disease is mediated by activation of a distal enhancer of *CARD9* leading to an increase of its transcription, supporting the causal role of *CARD9* in Inflammatory bowel disease²⁹. Similarly, there is increasing support to the notion that sQTL or mirRNA QTLs can also

contribute to common disease risk^{69,71,81}. For instance, the Multiple sclerosis associated variant rs6897932, located within an exon 6 of IL7R, has been shown to promote exon skipping. As a result, a soluble form of IL7R is being produced that is associated with exacerbated auto-immune phenotypes in mice⁷⁵. However, despite evidence that 90% of auto-immune loci act through regulatory variants, 60% of which are located at enhancers⁸², it was suggested that only a limited fraction of the QTLs detected at GWAS loci play a causal role in disease susceptibility^{63,83}. This observation, which may reflect the robustness of disease gene expression induced by the high redundancy of their enhancers⁸⁴, highlights the importance of taking linkage disequilibrium into account when assessing the co-localization between GWAS loci and eQTLs, or sQTLs⁸⁵.

Deciphering the regulatory code to predict the effect of rare variants

Recently, the development of massively parallel reporter assays (MPRA) has enabled the direct assessment of cis-regulatory activity associated to specific DNA fragments, either synthetic⁸⁶, or naturally occurring^{87,88}. The general principle of MPRA techniques (**figure 3**) consists in transfecting libraries of barcoded plasmids into live cells and counting the occurrence of each barcode in the transfected cells, as a measure of the activity of the associated regulatory sequence (after

normalization by the barcode frequency in the initial library). By measuring the activity of both alleles at heterozygous loci, these approaches further allow quantifying the impact of genetic variants, or haplotypes, from a single individual. Thus, they constitute a privileged tool for assessing the impact of rare variants on regulatory regions, or identifying causal variants among sets of linked SNPs identified through eQTL analyses^{88,89}. For instance, dissection of regulatory mechanisms at a 87 nucleotide-long *IFNB1* enhancer indicated that 83 substitutions, out the 261 possible, resulted in altered activity in virus-infected cells⁸⁸.

Massively parallel assays have also been used in the context of both splicing^{90,91} and miRNA-mediated regulation^{92,93}, revealing, for instance, that up to 16% of splice disrupting variants are located in deep intronic regions⁹⁰. With the development of deep learning frameworks for sequence-based predictions^{94,95}, data generated by these assays, will now fuel the construction of predictive models. These models will, in turn, allow quantifying the regulatory impact of novel genetic variants on transcriptional activity⁸⁸, alternative splicing⁹⁶, or miRNA-mediated regulation⁹³.

Concluding remarks: a strategy for the future.

Elucidating the mechanisms by which non-coding variants contribute to the inter-individual variability of immune response is now an essential step in order to

341 translate the findings of epidemiological genetic studies into the clinics¹³. The
342 characterization the human epigenome across a wide variety of tissues and
343 conditions has allowed establishing a first map of the regulatory landscape of the
344 human genome. Further efforts are now needed to fully define the cascade of
345 regulatory events that take place during the immune response, for a wide spectrum
346 of stimuli and with a high temporal resolution. This will allow classifying regulatory
347 elements according to their activity in distinct cell types, and in response to specific
348 stimuli. Then, using heritability partitioning techniques^{97,98} that decompose the
349 genetic variance of complex traits across several SNP categories, we will be able to
350 identify the cell types and stimuli that most strongly contribute to the development of
351 immune disorders. Holistic studies that consider both transcriptional and post-
352 transcriptional aspects of gene expression variation^{41,66,74}, and combine RNA seq
353 with sequencing of small RNAs or ATAC-seq, constitute a promising approach to
354 understand the aetiology of immune disorders. Such approaches, however, are only
355 the first step toward the identification of the causal mechanisms that underlie disease
356 susceptibility. Validation of the proposed mechanisms, through colocalization
357 analysis⁸⁵, mendelian randomization approaches⁹⁹ and randomized clinical trials¹⁰⁰,
358 are then required to formally establish causality. Finally, as whole genome
359 sequencing is becoming increasingly common¹⁹, the ability to identify rare regulatory

360 variants at known disease genes will become a crucial step toward personalized
361 treatments of both rare and common immune disorders. In this context, the
362 increased knowledge of the regulatory code granted by functional genomics studies,
363 and MPRA in particular, will constitute a precious tool to predict and assess the
364 effect of rare variants on intermediate disease phenotypes.

365

366

367 **Table 1. Epigenetic marks associated with gene regulation**

Epigenetic mark	Associated function	Assay
H3K4me1	Enhancers	Chip-Seq
H3K4me3	Promoters	Chip-Seq
H3K27Ac	Active regulatory regions (promoter/enhancer)	Chip-Seq
H3K9Ac	Active regulatory region (promoter/enhancer)	Chip-Seq
H3K36me3	transcribed regions /exon definition	Chip-Seq
H3K9me3	repressed region (heterochromatin)	Chip-Seq
H3K27me3	repressed region (polycomb)	Chip-Seq
Cytosine methylation	repressed regulatory regions (promoter/enhancer)	BisulfiteSeq
Open Chromatin	Active regulatory region (promoter/enhancer)	DNAse-Seq/ ATAC-Seq

368 **Table 2. Compared number of eQTL and sQTL detected across studies**

Study	Sample used	Number of eQTL detected	Number of sQTL detected	Metric used
Pickrell, 2010 ⁵⁶	lymphoblastoid cell lines (n=69)	929	187	Exon
Lappalainen, 2013 ⁵⁷	lymphoblastoid cell lines (n= 462)	3,773	639	Transcript
Battle, 2014 ⁵⁸	Whole blood (n=922)	10,914	2,851	Transcript
Nedelec, 2016 ⁶⁵	macrophages (n=175 x 3 conditions)	1,647	1,120	Transcript
Chen, 2016 ²⁹	Monocytes, neutrophils, CD4+ T-cells (n=197)	6513, 5845, 5799	3044, 2034, 3347	Transcript, Exon
Ye, 2018 ⁷¹	Dendritic cells (3 conditions, n= 99+227+250)	6,694	2,763	Transcript

Figure 1: The molecular bases of response-eQTLs.

a. Genomic dissection of a genic region. Grey arrow represents the transcription start site. Purple, green and orange lines represent possible chromatin-chromatin interactions between enhancers, or between enhancers and the promoter. Green/red circle represents a mutation of the orange enhancer. The same color

code is used throughout the figure. **b-c.** 3-dimensional representation of chromatin interactions leading to gene expression. At basal state (**b**), gene expression is mediated by the green enhancer, which comes in contact with the promoter (lightblue) and recruits the purple enhancer. Upon stimulation (**c**), the green enhancers is sequestered far from the promoter and replaced by the orange enhancer, allowing the mutation to alter gene expression. **d-e.** Activity of regulatory elements in the region, at basal state (**d**) and upon stimulation (**e**). Peaks indicate H3K27 acetylation with measures activity at promoters and enhancers. **f-g.** Boxplots of gene expression for each genotype, at basal state (**f**) and upon stimulation (**g**). Alleles at the locus are colour coded (red: low expression, green: high expression allele). The impact of the genetic variant located in the orange enhancer is visible only in the stimulated condition, where the enhancer is active.

Figure 2: Genetic regulation at the post-transcriptional level.

a. sQTL alter gene function by changing their splicing, leading to a different protein isoform. **b.** miRNA QTL that modify the abundance of miRNAs can act in *trans* in to alter gene expression of genes located elsewhere on the genome through miRNA-mediated degradation. **c.** SNPs that disturb (or create) miRNA binding sites in the 3'UTR of a gene can also change their level of expression, by acting in *cis* on miRNA-mediated degradation. Such SNPs are referred as reg-eQTLs.

Figure 3: Principle of massively parallel reporter assays (MPRA).

MPRA consists in forming libraries of putative regulatory sequences, where each sequence is associated to a specific, random barcode (1). The barcoded sequence is then introduced in a reporter plasmid, where it is ligated to the 3' of a green fluorescent protein (GFP) associated with a basic promoter sequence (2). The plasmid library is then transfected into a cell line representative of the tissue of interest (3). RNA from GFP positive cell are harvested and mRNAs of the reporter gene are captured and sequenced (4). The regulatory activity of each sequence is then measured by comparing the counts of its associated barcode in the transfected cell library with that of the initial sequence library (5).

411

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416

417 **Biosketch.**

418

419 Dr Maxime Rotival obtained his PhD in statistical genetics in 2011 from University of
420 Paris-Sud/XI. His PhD work regarded the genetic determinants of monocyte gene
421 expression in humans and their contribution to metabolic and cardiovascular
422 disorders. He did his first postdoc at Imperial College London, where he studied the
423 genetics of macrophage dysfunction in a rat model of chronic kidney inflammatory
424 disease, before joining the Human Evolutionary Genetics laboratory of Institut
425 Pasteur (CNRS UMR2000) in 2014. There he studied how genetic variants that alter
426 response to immune stimuli have contributed to the adaptation of modern human
427 population to pathogens present in their environment. He now has a researcher
428 position at institut Pasteur and is interested in the contribution of genetic diversity to
429 the inter-individual variability in innate immune response and how selective
430 pressures induced by pathogens have shaped this diversity. His work was awarded
431 with the Julia Bodmer Award for Young Scientists from the European Federation for
432 Immunogenetics in 2018.

433

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