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Genetic Variants in mRNA Untranslated Regions

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Abstract

Genome Wide Association Studies (GWAS) have mapped thousands of genetic variants associated with complex disease risk and regulating quantitative traits, thus exploiting an unprecedented high-resolution genetic characterization of the human genome. A small fraction (3.7%) of the identified associations is located in untranslated regions (UTRs), and the molecular mechanism has been elucidated for few of them. Genetic variations at UTRs may modify regulatory elements affecting the interaction of the UTRs with proteins and microRNAs. The overall functional consequences include modulation of mRNA transcription, secondary structure, stability, localization, translation, and access to regulators like microRNAs (miRNAs) and RNA-binding proteins (RBPs). Alterations of these regulatory mechanisms are known to modify molecular pathways and cellular processes, potentially leading to disease processes. Here, we analyze some examples of genetic risk variants mapping in the UTR regulatory elements. We describe a recently identified genetic variant localized in the 3′UTR of the *TNFSF13B* gene, associated with autoimmunity risk and responsible of an increased stability and translation of *TNFSF13B* mRNA. We discuss how the correct use and interpretation of public GWAS repositories could lead to a better understanding of etiopathogenetic mechanisms and the generation of robust biological hypothesis as starting point for further functional studies.

Graphical Abstract

Representation of human mature mRNA with indicated the main regulatory regions in the UTRs and the corresponding trans-acting factors.

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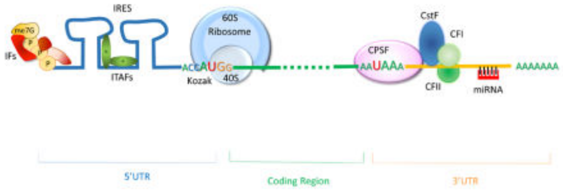

Introduction

In the last decade, Genome Wide Association Studies (GWAS) have uncovered many robust associations between genetic variants and risk of numerous complex diseases. The availability of high-quality genotyping microarrays[1–3] and the advent of large-scale human genome sequencing[4–7], and their integration using appropriate statistical methods, like imputation[10], have provided unprecedented high-resolution genetic profiles. These advances have allowed the analysis by GWAS of millions of genetic variants of the entire human genome with deep coverage.

However, only ~4% of the total genome-wide associated variants identified result in differences in the protein product, and in even fewer cases, the link between coding variant and mechanism contributing to the disease is apparent.[4]

Part of this discrepancy is likely due to problems inherent in the assessment of genetic variation. Although recent technological advances have enabled large-scale whole-genome sequencing (WGS) in some studies, most large GWAS have relied on DNA microarray (chip array) analysis, which only genotypes a subset of all genetic variation. In fact, microarray construction must balance numerous factors, like allele frequency, accuracy of genotyping and marker physical position, as well as purported functional role of the polymorphisms.[2] Likewise, the identification of the genetic variants themselves has not been a uniform, standardized search, but has instead been conditioned by the search method (expressed sequence tag searches, gene-region searches, etc), and also by the genotyped population, with some variants common in one human population being rare or absent in others.[6,7] As a result, a GWAS can provide the most-associated variant as the “best available marker”, rather than as the “probable causal variant”. Similarly, even in the case of WGS, non-random association of specific alleles at specific positions (known as “Linkage Disequilibrium” - LD),[8] complicates the problem of detecting the variant effectively associated with a disease or phenotype in a “haplotype block”.

Because of their easier interpretation, genetic variants in the coding sequence of a gene and present in the expressed coding regions (exons) have often been given priority, although it has long been clear that coding sequence variants *per se* were insufficient for mapping complex diseases.[9] However, variants in the intervening sequences (introns) or in the untranslated regions (UTRs), although not changing the predicted protein sequence, may be pivotal in the regulation of gene expression. The UTRs are the mRNA sequences flanking the beginning and end of the coding sequence; as their name suggests, UTRs are part of the mRNA but are not translated into protein. Notably, 3.7% of the genetic variants detected in GWAS studies are located in the UTRs.[11,12]

By convention, a genetic variant in the DNA sequence that occurs in a population with a frequency of 1% or higher is defined as "polymorphism" while the rarer ones (frequency <1%) are defined as "mutations".[13,14] Polymorphisms and mutations can comprise one or more nucleotide changes. Interestingly, mutations predominate in 5′UTRs, while polymorphisms are more common in 3′UTRs.[13,14] Gene expression is regulated at the RNA level by virtue of the presence of 5′ and 3′UTR regulatory elements such as upstream open reading frames (uORFs), internal ribosome entry sites (IRESs), as well as the UTR's secondary structure, sequence composition, and length. The majority of regulatory elements are recognized by RBPs or by non-coding RNAs (ncRNAs) such as miRNAs. Overall, these mechanisms modulate the mRNA stability, localization, and translation.[15,16]

Alteration of these regulatory mechanisms can modify molecular pathways and cellular processes, thus affecting phenotype, disease onset and possibly even disease outcome. In fact, genetic variations in the UTRs have been already implicated in several diseases such as melanoma, Alzheimer's disease, multiple myeloma, fragile X syndrome, bipolar disorder, breast cancer and other pathologies.[11]

In this review, we will describe the mechanisms of UTR regulation, and the role of genetic variants in modulating RNA processing and thus protein production in human disease. We will then give an overview of the GWAS results linked to UTR regions, and discuss an example of a genetic variant in the 3′UTR of *TNFSF13B* affecting the risk of autoimmune diseases and related immune phenotypes.[17] We conclude by presenting several open questions about UTR mechanisms.

REGULATORY ELEMENTS IN THE UNTRANSLATED REGIONS

Genetic variants at the 5′UTR

The 5′UTR is the RNA sequence immediately upstream of the coding RNA. It is generally not translated, although some exceptions, in which part of the 5′ UTR is translated, do exist.[18] In eukaryotes, its length ranges from a few nucleotides (nt) to several thousand, with an average in humans of about 200 nt.[16] The 5′ UTR should possess a Kozak consensus sequence (ACCAUGG), which contains the translation initiation codon. It may also contain numerous regulatory elements, like CpG sites, uORFs, IRESs, and RBP binding sites, which will be treated later. Additionally, secondary structures, such as hairpin loops, may be important in translation regulation, and often occur within the 5′UTR. Thus, genetic variants modifying these regulatory elements can have important impact on the overall production of the protein by affecting RNA transcription, stability, and translation.[16,19, 20]

In the next sections, we will describe the regulatory elements at the 5′UTR and give some examples of genetic variants affecting their function.

5′UTR length, CpG sites and Kozak sequence—Genes with differences in 5′UTR length are relatively common mainly due to the presence of multiple promoters[21] or alternative splicing mechanisms within UTRs[22] and may have clinical effects. For example, deletion in the 5′UTR of the ATPase copper transporting beta, ATP7B, reduces the activity of the *ATP7B* promoter resulting in less protein and increased Wilson disease predisposition.[23]

Besides length *per se*, CpG sites, repeats of cytosine followed by guanine, are often present in 5′UTRs. These regions can undergo cytosine methylation, an epigenetic modification that promotes gene silencing by recruiting proteins involved in gene repression of by inhibiting the binding of transcription factors.[24] The higher the number of CpG sites in a 5′UTR, the higher the probability that the gene expression will be downregulated as a consequence of cytosine methylation. For example, fragile X syndrome, a genetic disorder characterized by several intellectual disabilities, is caused by the expansion of a CGG-repeat sequence in the 5′UTR of the *FMR1* gene. Expansion to >200 copies of the CGG-repeat leads to hypermethylation of FMR1 and silencing of this gene, resulting in an insufficient amount of fragile X mental retardation protein that is pivotal for neuronal development.[25]

Other mutations in specific regulatory elements, such as the Kozak consensus sequence[26] can have an important impact on protein production. For example, mutation in the Kozak sequence of the β-globin gene leads to a 30% reduction of the translational rate of the beta-globin gene, while not altering the transcription level.[19]

Open reading frames—The ORF is defined as the part of a reading frame that has the potential to be translated; it consists of a sequence of nucleotide triplets that specify an amino acid chain. While the mRNA of a gene will have a principal ORF that specifies the main polypeptide product, there may be several other ORFs, each of which modulate the overall expression of the main protein product. The ORFs located upstream to the canonical initiation codon and out-of-frame with respect to the main coding sequence are called upstream (u)ORFs and are characterized by their own upstream starting codon (uAUG) and stop codon. At least half of the human transcripts contain uORFs. They correlate significantly with reduction of protein expression (30–80%) of the downstream ORF, with only a modest impact on mRNA levels.[27] The uORF-mediated translational control can occur through different mechanisms, depending on the efficiency of uAUG ribosome recognition and of uORF translational termination.[28] The uORFs can be generated or disrupted by genetic variants leading to dysregulation of gene expression and increased disease risk.[27,29]

For example, the creation of a new uORF was observed in the *SPINK1* gene. This gene encodes a trypsin inhibitor, Serine Peptidase Inhibitor, Kazal Type 1, that prevents the activation of zymogens within the pancreas.[30] A mutation (C>T) at position -53 to the main AUG start codon generates a new AUG start codon and a uORF that dampens the efficiency of Spink1 translation, leading to hereditary pancreatitis.

The disruption of uORFs are also frequent in nature, for example a point mutation in the second uORF of the gene encoding the human hairless homolog (HR) causes the elimination of the ATG codon, leading to the absence of the corresponding 34 amino acid peptide, which has a negative regulatory effect on the main HR. In this way, the variant causes an increased translation of HR and Marie Unna hereditary hypotrichosis, an autosomal dominant form of genetic hair loss.[31]

Other mechanisms involving mutations in the uORF can affect the expression of the corresponding encoded peptide, predisposing to disease. For example in bipolar disorder, the missense mutation C178T in the uORF reduces the repressive activity of the uORF encoded peptide (P16S amino acid change), causing an increase in HT3A, a receptor subunit involved in neuronal depolarization.[32]

Internal Ribosome Entry Sites—Translation initiation is a complex event requiring several proteins, called initiation factors (IFs), which allow i) the formation of the ribosomal pre-initiation complex, ii) the recruitment of the 43S complex to the 5′ end of the mRNA, iii) the scanning of the 5′UTR, and iv) the recognition of the AUG codon and the 5′ cap. Capping at the 5′ end is a key process consisting in the addition of 7-methylguanosine to the 5′UTR, which confers protection to the mRNA against degradation, ultimately promoting nuclear export and translation.[33]

Internal ribosome entry sites (IRESs) are RNA elements that allow the translation initiation in a cap-independent manner by recruiting the ribosome to the mRNA for protein synthesis. Discovered in 1988 in the poliovirus RNA genome,[34] IRES are characterized by several elements recognized by proteins involved in translation, such as IRES trans-acting factors (ITAFs), but also canonical initiation factors. IRESs are present in many viruses and in eukaryotic mRNAs involved in responses to stress conditions (hypoxia, heat shock, nutrient limitation) or in response to signals to survive, differentiate, proliferate, or undergo apoptosis.[35]

IRES mutations can alter protein expression and cause disease. For example, the 5′UTR of the proto-oncogene c-MYC contains an IRES and, in patients with multiple myeloma, a C>T substitution within the IRES causes increased synthesis of c-MYC protein by favouring the binding of two ITAFs, Y-box binding protein 1 (YB-1) and polypyrimidine tract-binding protein 1 (PTB-1).[36] Another example is represented by a mutation in the IRES of the connexin-32 gene, which abolishes translation of the corresponding mRNA in nerve cells, leading to Charcot-Marie-Tooth disease, a neurodegenerative disorder.[37]

RNA-binding proteins and 5′UTR regulation—RBPs are key components of the ribonucleoproteins complexes (RNPs) which modulate gene expression by binding the mRNA molecules and may act in both the cytoplasm and the nucleus. RBPs regulate several phases of co- and post-transcriptional gene expression, such as RNA capping, splicing and polyadenylation, and mRNA export, localization, stability and translation.[38]

Binding of RBPs to RNA targets is mediated by a set of modular RNA-binding domains, such as the RNA recognition motif, heterogeneous nuclear RNP K-homology domain, and zinc fingers,[39] while the RNA target is characterized by short, single-stranded (ss)RNA sequences, often having specific secondary structures.[40]

Functional genetic variants in the RNA targets can affect RBPs recruitment by generating or disrupting their binding sites, as well as by modifying the RNA secondary structure.

Tryptophan hydroxylase (TPH) is an enzyme responsible for neuronal serotonin (5-HT) synthesis and it is encoded by two genes, *TPH1* and *TPH2*. While *TPH1* is primarily expressed in the periphery, *TPH2* is predominantly expressed in the brain. Polymorphisms in *TPH2* are associated with a range of behavioural traits and psychiatric disorders.[41,42] Chen and colleagues demonstrated that the 90 A/G polymorphism at the 5′UTR of the *THP2* mRNA alters the mRNA structure and/or RNA–protein interaction, thus affecting *TPH2* gene expression at the post-transcriptional level.[43]

The amyotrophic lateral sclerosis-associated RNA-binding protein (TDP-43) was linked to the pathogenesis of fragile X-associated tremor/ataxia syndrome (FXTAS). In FXTAS, a CGG repeat expansion in the 5′UTR of the *FMR1* gene caused a progressive neurodegeneration in human patients. In a drosophila model of FXTAS, He and colleagues identified TDP-43 as the suppressor of the CGG-induced toxicity, although it required two heterogeneous nuclear ribonucleoproteins (Hrb87F and Hrb98DE) for its activity. In fact, deletions in TDP-43 that prevented the interaction with the two ribonucleoproteins nullified the beneficial effect of TDP-43 function of CGG-repeat toxicity. These results suggest a model in which the repeat expansion of CGG at the 5′UTR and the modified interaction with a RBP are implicated in neurodegenerative disease.[44]

Introns at 5′UTR—Approximately the 35% of human genes contain introns in the 5′UTR.[45] These 5′ introns are less common than introns within coding regions, but are, on average, longer.[46] By analysing the expression profiles of genes with 5′UTR introns, Cenik and colleagues found that the most highly expressed genes reveal a strong enrichment of short 5′UTR introns with respect to long or absent 5′UTR introns.[18] No relationship was found between length and expression level for genes with intermediate or long 5′ introns. Considering that expression depends on production and degradation rates of mRNAs, Cenik's results suggest that short 5′UTR introns tend to increase transcription or stabilize mature mRNAs.

Genetic variants at the 3′UTR

The 3′UTR is located downstream of the coding sequence, and it is involved in regulatory processes, including RNA stability, mRNA translation and localization. The 3′ UTR is characterized by binding sites for RBPs and miRNAs, and thus any variation in the 3′UTR length and sequence may change the binding for miRNAs and RBPs, leading to change in gene expression.

3′UTR length and alteration of the polyadenylation signal—The importance of 3′UTR length in mRNA stability, translation, tissue-specific expression, timing and function is demonstrated by several studies in health[47–49] and disease.[50,51] For example, in a recent work Romo and colleagues showed that the huntingtin gene (HTT) is characterized by three mRNA isoforms, two of which had different 3′UTR lengths. The amount of the two 3′UTR isoforms differed between Huntington disease patients and controls; moreover, while the longer isoform is more represented in the neuronal precursor cells, breast and ovary, the shorter isoform is more prevalent in testes, B cells and muscle, and the abundance of HTT isoforms changes in a tissue-specific manner in Huntington patients.[52]

One important mechanism causing alteration in the 3′UTR length is the modification of the polyadenylation signal. Like the 5′ cap on mature mRNA, the stability, nuclear export and translation is also regulated by a stretch of adenine nucleotides that are added at the 3′end of the mRNA by specialized enzymes. The cleavage and polyadenylation specificity factor (CPSF) recognizes the specific sequence AAUAAA on the pre-mRNA and cuts 3′UTR about 10–30 nucleotides downstream of its binding site. Other proteins, such as the cleavage stimulation factor (CstF) and cleavage factors (CFI, CFII) work in concert creating, together with CPSF, the RNA-cleavage complex. Once the RNA is cut, the polyadenylate polymerase adds adenosine monophosphates creating the poly-A tail.[47]

Several genes contain multiple polyadenylation sites which, by changing the length of the 3′ untranslated regions, may alter the number of binding sites for miRNAs and RBPs, thus modifying protein expression patterns and influencing disease. An example is represented by a point mutation in the canonical poly-A signal (AAUAAA→AAUGAA) of the forkhead box P3 gene (FOXP3), highly expressed in regulatory T cells. The mutation reduces the protein expression and causes a rare autoimmune disease, named immune dysfunctions polyendocrinopathy enteropathy X-linked, also known as IPEX syndrome.[53] Similarly, mutations in the poly-A signal of α- and β-globin genes cause a decreased production of the corresponding proteins resulting in thalassemia.[54–56] Conversely, the introduction of a novel alternative poly-A signal can dysregulate the protein production, leading to increased risk for disease. An example is represented by the recently discovered variant, associated to both multiple sclerosis and systemic lupus erythemathosus, which will be described below.[17]

MicroRNAs and miRNA binding sites—MicroRNAs are small non-coding RNAs acting as regulatory elements in gene expression.[57] miRNAs are transcribed as primary miRNA (pri-miRNA) and then cleaved by a nuclear complex, including the Drosha and Pasha proteins, resulting in the production of a precursor miRNA (pre-miRNA).[58–60] Pre-miRNAs are then exported to the cytoplasm by exportin-5 and cleaved by the Dicer enzyme, yielding a double-stranded (ds) miRNA.[61] Finally, miRNAs are loaded in the RNA-induced silencing complex (RISC) to suppress stability and/or translation of the mRNA target.[62] miRNAs recognize and bind miRNA Regulatory Elements mostly located in the 3′UTR of target mRNAs;[57] however miRNAs binding other regions, such as 5′UTR, have also been described.[63]

After the discovery of miRNAs, polymorphisms affecting miRNA function were identified by several approaches. Modern bioinformatic and statistical analyses, such as GWAS, combined with RNA sequencing and CLIP (cross-linking immunoprecipitation) data, represent key tools in the identification of genetic variants of miRNA binding sites and their impact on gene expression.

Functional variants can be divided into two groups, depending on whether they generate or disrupt miRNA binding sites in target mRNA.[64,65] Additionally, Single Nucleotide Polymorphisms (SNPs) and genetic variants in general can modify the secondary structure of the mRNA by affecting the accessibility to binding sites, or by altering the presence of the miRNA-binding site in the mature mRNA.[66,17]

To study polymorphisms affecting miRNA-binding sites, compared to SNP located in others regions, Lu and colleagues analysed the genotype and the mRNA expression in four populations, as part of the international HapMap Project. They found that compared to introns, 3′UTRs contain higher numbers of SNPs associated with changes in mRNA expression levels.[67]

Using SNP data, including those from the 1,000 Genomes Project, Richardson and colleagues performed a genome-wide scan of SNPs that disrupt or create new miRNA recognition element site. Specifically, the authors identified 2,723 SNPs disrupting, and 22,295 SNPs creating new miRNA binding sites. Additionally, by analysis of co-expression and eQTL data, they also identified four SNPs with a clear functional role. Among them, rs907091, localised in the *IZKF3* gene, a transcription factor important for B-cell activation, created a new binding site for mir-326 with a potential role in autoimmune diseases.[68]

The correlation between genetic variants at the 3′UTR and miRNA function has been extensively studied and are often associated with diseases. In 2016, Ghanbari and colleagues performed an analysis to identify genetic variants in miRNA genes and in miRNA-binding sites associated with Alzheimer Disease (AD). They found 237 variants in 206 miRNA genes and 42,855 variants in miRNA-binding sites present in AD-GWAS.[69] Among the 42,855 variants located in the miRNA-binding sites, they found 10 of them located in the 3′UTR of nine genes, including rs6857, which is predicted to create a target site for miRNA-320e in the 3′UTR of the poliovirus receptor-related 2 (*PVRL2*) gene.[69]

As SNPs perturbing miRNA-mRNA regulation can induce aberrant expression of autism-related genes, Vaishnavi and colleagues developed a systematic computational pipeline that integrates data from established databases. Using stringent selection criteria, they were able to identify 9 SNPs modulating and 12 creating new miRNA-mRNA regulation in the 3′UTR of autism-associated genes.[70] This paper provides valuable candidate SNPs affecting autism pathogenesis but unfortunately, as for other studies, further functional experiments are needed to validate the predicted data.

Furthermore, in a recent study, Zhang and colleagues identified a genetic variant (rs61764370) localized in the 3′UTR of the Kirsten rat sarcoma viral oncogene homolog (*KRAS*) that interfered with miRNA/mRNA interaction, and increased risk of developing metastasis in osteosarcoma.[71] Using several approaches, the authors demonstrated that the SNP interferes with the interaction between 3′UTR of *KRAS* mRNA and the miRNA let-7a, thus increasing KRAS protein level and influencing disease outcome.[71]

Together these studies demonstrate the relevance of dissecting genetic variants in the 3′UTR, particularly those involved in the interaction between miRNA and mRNA, and highlight the importance of genetic variants located in miRNA-binding sites in human diseases.

RNA-binding proteins and 3′UTR regulation—Genetic variants that modify the binding sites of RBPs in the 3′UTR can influence mRNA stability, translation efficiency and localization, by affecting the RNA-binding sequence and domain.

AU-rich elements (AREs) are RNA-binding domains recognized by certain RBPs, such as Human antigen (Hu) R (HuR), and ARE/poly(U)-binding/degradation factor 1 (AUF1), both implicated in controlling mRNA stability.[72,73] AREs are present in the 3′UTR of TNF mRNA and modulate TNF production at post-transcriptional level. Di Marco and colleagues showed that two polymorphisms (GAU and CAU trinucleotide insertions), localized in the 3′UTR of TNF mRNA, affect the binding of RBPs, with consequent reduction in the TNF protein expression a mouse model. Importantly, they showed that the polymorphism reduced HuR binding affinity to the ARE, thereby decreasing the production of TNF protein in macrophages.[74]

Another example is represented by the *PPP1R3* gene, encoding the muscle-specific glycogen-targeting regulatory PP1 subunit, which is involved in the regulation of glycogen synthesis in skeletal muscle. Xia and colleagues identified a polymorphism (ARE) in the 3′UTR of the *PPP1R3* gene, which reduces the distance between two mRNA-destabilizing sequence ATTTA. The polymorphism is characterized by a 10-nucleotide (allele ARE1) versus a 2-nucleotide interval (allele ARE2). Interestingly, ARE2 was associated with insulin resistance, increased prevalence of type 2 diabetes and reduced expression of this PPP1R3 subunit, causing a reduction in the half-life of the corresponding mRNA. Three proteins of 43, 80, and 139 kDa seem to bind the polymorphic ARE region and the less stable ARE2 allele shows higher protein binding, suggesting the role of the ARE2 in reducing mRNA stability.[75]

A complex polymorphism, a 6-nucleotide insertion/deletion in the 3′-untranslated region of the thymidylate synthase (*TS*) gene, affects mRNA stability by modulating the binding of AUF1 to *TS* mRNA. Pullmann and colleagues demonstrated that AUF1 has higher affinity for the deletion in the 3′UTR *TS* mRNA, consequently rendering it less stable, compared to the insertion in the same site. Additionally, they demonstrated that AUF1 overexpression preferentially suppressed the deletion allele.[76]

These studies demonstrate the importance of the identification of genetic variants at the 3′UTR affecting RPBs, as potential predisposing factors for complex diseases, their course, prognosis and complications.

Introns in 3′UTRs—Analogous to what was found in the 5′UTR, also the presence of an intron in the 3′UTRs may influence gene expression. 3′UTRs are generally much longer than 5′UTRs, but relatively few 3′UTRs (<5%) contain introns.[46] The reason could be partially explained by nonsense-mediated decay, by which transcript degradation would be typically signalled by an intron downstream of the stop codon.[77] In addition, splicing signals within 3′UTRs have been suggested to have reduced maintaining selection, being the 3′UTRs better able to tolerate loss of intron integrity than other gene regions; consequently, 3′UTRs tend to be longer with fewer introns compared to 5′UTRs.[78]

RiboSNitches, structural variation and RNA regulation

RNA folding to a specific conformation represents an essential step for the function of mRNAs. Structured elements in the UTRs of specific mRNAs can control gene expression and consequently affect physiological processes and disease onset. Today, the extent to which RNA conformational modifications impact the RNA function is still largely unexplored. RNA secondary structure (RSS) differences may have profound implications not only regarding RNA stability, protein binding and translation, but also in disease predisposition and personalized medicine.

RiboSNitch are Single Nucleotide Variant (SNV), found in the UTRs of mRNA transcripts as well as in ncRNAs, that alters the secondary structure of an RNA transcript.[79] They are analogous to bacterial riboswitches – RNA elements that adopt a different conformation after binding specific small molecules, leading to gene expression changes.[80,81] With riboSNitches, it is the base changes, rather than the binding of a small molecule, that promote RSS rearrangements.[82] Experiments have suggested that riboSNitches are not isolated peculiarities: astudy of RSS in a human family trio, identified more than 1900 transcribed variants, corresponding to 15% of all transcribed SNVs that could alter local RNA structure and hence the “RNA folding landscape”.[83]

To predict the impact of a genetic variant in RNA conformation, several algorithms have been developed.[84,85] For example, applying the SNPfold algorithm to all known disease-associated SNPs from the Human Gene Mutation Database, and mapping in the UTRs, Halvorsen and colleagues identified 6 diseases (hyperferritinemia cataract syndrome, β-thalassemia, cartilage-hair hypoplasia, retinoblastoma, chronic obstructive pulmonary disease, and hypertension) where multiple SNPs, in the UTRs of disease-associated genes were predicted to cause RNA conformational change.[79]

By changing the RNA conformation, a riboSNitch can alter the binding of RBPs and miRNAs that interact with the transcript. The interaction between iron responsive element (IRE) and IRE-binding protein (IREBP) in the ferritin light chain (FTL) RNA, requires both a correct IRE sequence and an exact RNA conformation. Both elements allow the RNA interaction with IREBP, which ultimately lead to translational repression. Mutation in any residue that shifts the structure of the IRE, is able to prevent IREBP binding, leading to increased FTL translation and hyperferritinemia phenotype.

Several molecular biological techniques have been developed to interrogate RNA structure at single-nucleotide resolution, including SHAPE.[86] This technique was applied to show that the correct FTL conformational structure can be restored by a group of SNPs in LD, namely a structure-stabilizing haplotype (SSH). This may explain some cases of strong LD between SNPs and also indicate a set of “causal SNPs”, rather than a single “causal mutation” for some phenotypes. Moreover, a comprehensive analysis of human genetic variation highlighted that SSHs are common in mRNA and they generally stabilize the RBP target sites.[87]

Similarly, using SHAPE, Kutchko and colleagues identified 3 different functional conformations of the 5′UTR of retinoblastoma 1 (RB1), also finding that private SNVs in two patients with retinoblastoma caused the collapse of the RNA structural ensemble, leading to a specific RNA abnormal conformation.[88]

Beyond retinoblastoma, riboSNitches have also been found in the *H19* gene, a long non-coding RNA involved in several cancers. Li and colleagues observed that rs2839698 GA/AA genotypes increase the risk of colorectal cancer in the Chinese populations compared with the GG genotype. Interestingly, the A allele generates an important conformational change in the folding structure of H19 that may cause the loss of the target binding site for some miRNAs, while creating a binding site for other miRNAs.[89]

A further example of a pathogenic RiboSNitch and its potential use in personalized medicine is represented by the SNP rs12455792, localized in the 5′UTR of the *SMAD4*, a gene involved in blood vessel remodeling and matrix maintenance. Wang et al. demonstrated that the CT or TT genotypes were associated with reduced transcriptional activity, altered RNA folding structure, and decreased SMAD4 expression, as well as significantly elevated risk of thoracic aortic aneurysm and dissection (TAAD). Moreover, using computational analysis and other approaches, they showed that the lower SMAD4 expression might be due to a reduced function of a RNA hairpin structure. Additionally, *SMAD4* mRNA abundance, assessed in freshly frozen aorta tissues from TAAD patients, was significantly higher in CC genotype than in CT or TT genotypes, suggesting rs12455792 as a predictor of TAAD progression.[90]

Overall, these findings indicate that riboSNitches are an exciting and active research area and likely represent an important set of genetic variants, the characterization of which should ultimately be very useful in identifying causal variants, both in the UTRs and beyond.

Nonsense-mediated decay

Another way that polymorphisms in UTRs can influence gene expression is through nonsense-mediated decay (NMD) of mRNA. NMD is a safeguard mechanism that prevents cells from generating deleterious truncated proteins. It degrades abnormal mRNAs that contain a premature termination codon (PTC). NMD can also target normal, non-mutant, transcripts thus regulating gene expression and impacting several physiological processes such as cell differentiation, response to stress, neuronal development, and the onset of various diseases.[91] Aberrant splicing, long 3′UTR and uORF are some of the mechanisms implicated in NMD activation.[92]

An illustrative example with important ramifications is AUF1, which targets mRNAs containing AU-rich elements (AREs) for rapid cytoplasmic turnover. Alternative splicing generates five variants of AUF1 mRNA, which have different 3′UTRs. The generation of alternative 3′UTR can affect AUF1 expression by two mechanisms: AUF1 protein directly binding AUF1 3′UTR splice variants that retain intron 9 (affected by the alternative splicing), and activation of the mRNA NMD pathway. Two of the AUF1 3′UTR variants position the translational termination codon more than 50 nucleotides upstream of an exon-exon junction, creating a potential triggering signal for NMD. Disruption of cellular NMD pathways by gene specific knockdown enhanced the mRNA expression of these two AUF1 isoforms, with stabilization of each transcript. Additionally, quantification of AUF1 mRNA 3′UTR splice variants during murine embryonic development showed that the expression of NMD-sensitive AUF1 mRNAs is specifically enhanced as development proceeds, contributing to dynamic changes in AUF1 3′UTR structures during embryogenesis.[93]

Using microarray analysis, Kim and colleagues revealed that the level of cyclin-dependent kinase inhibitor 1A (CDKN1A; also known as Waf1/p21) mRNAs increases in cells depleted of cellular NMD factors. Interestingly, p21 mRNA contains an uORF, which is a NMD-inducing feature. Using several approaches, they identified the uORF in *CDKN1A* mRNA as a negative modulator of translation of the main downstream ORF, thus providing additional biological evidence of the possible role of NMD in diverse biological pathways.[94]

NMD has been implicated in the onset of several diseases; for example, NMD-induced loss-of-function was shown to contribute to the onset of certain cancers. Hu and colleagues developed an algorithm to predict NMD and applied it to somatic mutations, finding 73,000 mutations that are predicted to elicit NMD and are associated with significant reduction of gene expression in tumour suppressor genes.[95] Interestingly, half of the hypermutated stomach adenocarcinomas are characterized by NMD-eliciting mutations in two genes implicated in translation initiation (LARP4B and EIF5B). Together these results underline the key role of NMD in human pathophysiology.

WHAT IS KNOWN FROM GWAS STUDIES

GWAS data are increasing rapidly, and thus the scientific community needs to develop appropriate tools to manage systematically the large amount of information available. With this goal in mind, several databases collecting GWAS data related to diseases and quantitative parameters have been developed.

For example, the NHGRI GWAS Catalog,[12] is a quality-controlled, manually curated collection of all those published genome-wide association studies which assay at least 100,000 SNPs. The NHGRI GWAS Catalog contains all SNP-trait associations with p-values $< 1.0 \times 10^{-5}$.[11]

With the aim to evaluate the distribution and impact of variants at UTRs, we extracted data from a recent version of this catalog (www.ebi.ac.uk/gwas; accessed 2017-09-19, version v1.0).

As all the reported variants in the GWAS Catalog are annotated based on their genetic position, we estimated that the 3.7% of the 57,671 variants included in this dataset are localised in the UTRs. In particular, 1,652 of them map in the 3′UTR, representing 2.9% of the total variants, while 442 are in the 5′UTR, reaching the 0.8% (table 1).

Thus, almost 80% of the UTR associated variants reported in the literature are localised in the 3′UTR region. However, as the 3′UTRs are, on average, much longer than 5′UTRs, we adjusted the number of identified variants per the UTRs length, observing a comparable number of associated variants in both UTRs (table 2). These estimations were obtained by exploiting genomic data from the Ensembl database (http://grch37.ensembl.org/biomart/, database: Ensembl Genes 91, dataset: Human Genes - GRCh37.p13).

Moreover, taking advantage of this catalog, we searched for variants mapping the UTRs that affect disease risk and/or quantitative parameters. For instance, among diseases, genetic variants at UTRs are more frequently associated with immunological, neoplastic and neurological pathologies (figure 1, panel A). Similarly, among the quantitative traits, we observed that the large part of known UTR associations were involved in immune-related, haematological, anatomical parameters and metabolic traits, while a small number of variants affect inflammatory and cardiological phenotypes (figure 1, panel B).

Furthermore, we selected the strongest and most common associations mapping in the untranslated regions; in particular, we extracted 15 disease-related and 35 trait-related SNPs, whose p-values represent the top 5% percentile for each category, considering a p-value threshold lower than 5×10^{-8} and a minor allelic frequency - MAF > 5% (table 3 and table 4).

Among the SNPs associated with diseases (table 3), we cite some examples mapping in 5′UTRs, and showing a moderate impact (expressed as odds ratio) in cancer or autoimmune pathologies. For example, Tanikawa and colleagues found the SNP rs2294008 to be associated with duodenal ulcer in the *PSCA* gene, encoding prostate stem cell antigen. This gene is a good candidate, being highly expressed in several tissues, such as bladder, placenta, colon, kidney, and stomach, and also being detected in pancreatic and bladder cancers. The SNP risk allele for the disease encodes a translation initiation codon upstream of the reported site, thus changing protein localization from the cytoplasm to the cell surface.[96]

Another interesting variant that recently emerged from GWAS is rs2189521, associated with primary biliary cholangitis (PBC) in the IL21 receptor gene (*IL21R)*.[97] Qiu and colleagues reported that the risk allele for PBC regulates differential IL21R expression; this variant is also highly correlated with multiple SNPs in the *IL21R* region, suggesting that variation in *IL21R* expression may explain this signal. By applying several histochemical experiments, they showed that the enhanced expression in PBC livers (in the hepatic portal tracks) of *IL21R* and of its ligand, *IL21*, support an involvement of IL21 signalling pathway deregulation in the disease mechanism.

Likewise, among SNPs mapping in the 3′UTR, one of the strongest associations is the SNP rs6427196, localized in the coagulation factor V gene (*F5*) and found associated, in a large meta-analysis, with venous thromboembolism (odds ratio = 2.07, p-value = 4×10^{-51}).[98]

Another variant with a strong effect (odd ratio = 2.26, p-value = 2×10^{-50}) is rs995030, associated with testicular germ cell tumour in *KITLG*, encoding the ligand for the receptor tyrosine kinase KIT.[99] The KIT–KITLG system regulates the survival, proliferation and migration of germ cells, and mutations in this gene confer an increased tumour risk in a mouse model of the disease. Although the gene may explain the association, no correlation has been found so far between rs995030 and variation in *KITLG* expression.[100]

Among the strongest association of genetic variants in UTRs with quantitative traits (table 4), the following two examples are particularly informative.

Variants in the apolipoprotein A5 (*APOA5*) gene are associated with lipids levels, mainly HDL cholesterol, and with related dysfunctions including the metabolic syndrome. Several of these variants map in UTRs, such as the SNP rs651821 localized in the 5′UTR region.[101] By searching this SNP in a recently published catalog of gene expression data (https://www.gtexportal.org/home/),[102] we observed that it is an eQTL (in the adipose –

subcutaneous specific tissue) for a long non coding RNA gene (RP11-109L13.1) located 400 kb downstream the *APOA5* gene. The effective implication of this gene in lipid modulation is suggested by a second variant mapping in the 3′UTR of the gene and showing pleiotropic effects on triglycerides and HDL-C levels: the SNP rs2266788.[103] Interestingly, in a more recent work, Caussy and colleagues showed that the less frequent allele of rs2266788, belonging to *APOA5* haplotype 2 (APOA5*2), reduces APOA5 expression at the post-transcriptional level by creating a functional target site for miRNA485-5p, mainly expressed in the liver. Therefore, the increased level of triglycerides in the presence of APOA5*2 could be caused by the APOA5 downregulation mediated by miRNA485-5p.[104]

After genetic variants associated with diseases or parameters have been identified, it is important to establish their specific gene localization and, consequently, their functional effects. To this end, several tools, such as the Ensembl Variant Effect Predictor (VEP),[105] are now available. However, since the localization of a variant may be different in different isoforms of the same gene, the variant can be predicted to map in UTRs (in one isoform) as well as in introns, in non-sense mediated decay (NMD) or non coding transcripts, in regulatory regions, in transcription factors (TF) binding sites or even outside the gene (in alternative isoforms) (figure 2). This introduces an extra layer of complexity in genetic data interpretation.

An important tool to estimate the detrimental effect of a variant is represented by the Combined Annotation Dependent Depletion score (C-score).[106] This score combines different information, such as the variant consequence on DNA gene sequence, its impact on expression, acetylation, and methylation, and the conservation score of the region, in a single metric. The higher the C-score, the more deleterious is the variant; to identify potentially pathogenic variants, Kircher and colleagues suggested using a cut-off value between 10 and 20. In this way, the C-scores give important information about the different allelic impact of a variant, its functional role and pathogenicity; also allowing to rank causal variants in a genome sequence. We calculated the C-score for UTR variants in the GWAS Catalog data and obtained values ranging from 0.001 to 22.1, with a relatively low mean value (mean=5.89), indicating that variants in UTRs are mainly benign. We then prioritized only those variants unequivocally mapping in UTRs from VEP annotation (table 5). Among the most deleterious variant, rs1128334 (C-score = 16.2) was notable in that it was associated with systemic lupus erythematosus risk and was located in the 3′UTR of the *ETS1* gene. *ETS1* encodes the transcription factor C-ets-1, involved in a wide range of immune functions, including Th17 cell development and terminal differentiation of B lymphocytes. When evaluating allelic expression in peripheral blood mononuclear cells, the risk allele showed lower *ETS1* expression levels.[107]

An example of 3′UTR genetic regulation predisposing to autoimmunity

Since the early 1900s, it was postulated that a qualitative condition such as the presence/absence of a given pathology could be caused by multiple quantitative traits, each of which is influenced by a number of genetic variants.[108] This model fits particularly well into the context of complex traits and common diseases in which many variants with small effect are involved in disease predisposition.[109] In this context, the study of quantitative trait variation is a valuable approach to dissect the predisposition to complex diseases through the analysis of the biomedical parameters in population cohort individuals without the use of case-control strategies that rely on differences between patients vs healthy individuals. The dissection of quantitative trait variation in the general population shows several advantages including the large sample size and the collection of raw data unaffected by the pathology itself or by the drug treatment. This will increase the accuracy and robustness of data and thus will harness the power to detect associated variants in GWAS studies.

The resulting genetic association data of quantitative traits will then be compared with case-control studies for pathologies in order to identify those genetic variants associated with both a quantitative trait and a disease. This approach can reveal disease-related endophenotypes, thus helping on one hand to identify causal variant(s) at a locus and, on the other hand, to elucidate disease etio-pathogenesis.

By applying this approach to autoimmune diseases and immune-related quantitative parameters, we recently identified a genetic variant localized in the 3′UTR of the *TNFSF13B* gene that increased the stability and translation of the corresponding mRNA, and having a pivotal role in autoimmunity.[17]

TNFSF13B encodes the protein BAFF (B-cell activating factor), a cytokine primarily produced by monocytes and neutrophils, and involved in the development, survival and differentiation of B cells.[110,111] The variant predisposing to autoimmunity results from the combination of a deletion with a polymorphism (GCTGT>A), referred to as BAFF-var. BAFF-var generates an alternative polyadenylation signal, leading to a mixed population of mRNAs characterized by long and short 3′ UTRs, in contrast to the wild-type allele, which produces only a long 3′UTR transcript. We observed that individuals carrying one copy of BAFF-var had a 35% reduction of the long transcript. Notably, the production of the shorter 3′UTR transcript was responsible for an increase of the RNA stability and translation due to the absence of a miRNA binding site for miR-15a, resulting in a rise of soluble BAFF. However, the increase in the mRNA expression explained only about 24 to 27% of the higher amount of soluble BAFF, indicating that an increase in translation level was also probably involved. The strong increase in the serum concentration of the soluble BAFF protein (about 19% per BAFF-var allele) led to other important downstream events closely linked to BAFF function, such as a rise of the number of circulating B cells and immunoglobulins, mainly IgG, IgA and IgM. Additionally, we observed a reduction of circulating monocytes, a phenomenon probably due to a negative feedback mechanism to compensate the augmented production of soluble BAFF in these cells. Overall, the immune system dysregulation caused by BAFF-var led to increased risk of multiple sclerosis and systemic lupus erythematosus (figure 3).

Interestingly, BAFF-var is particularly frequent in Sardinia (26.5%), where the main study was conducted, with decreasing frequency when assaying from Southern (5.7%) to Northern European populations (1.8%). This high allele frequency in Sardinia allowed us to identify the association with multiple sclerosis at a genome-wide significance level using a relatively small sample set of about 3,000 patients and a similar number of controls.

A possible explanation of the high frequency of BAFF-var is its positive selection due to a selective pressure acting in Sardinia and, in general, in Southern Europe. The most plausible candidate is malaria infection, as suggested by the correlation between the frequency of this variant vs malaria prevalence across Europe before its eradication (~1950).[112] Additionally, BAFF transgenic mice survived lethal *Plasmodium yoelii* malaria, and there is evidence that the malaria parasite can prevent long-term immunity dysregulating the dendritic cells producing BAFF.[113]

This is a classic example of hygiene hypothesis, in which a genetic variant for a long time positively selected because protective for an infectious disease, predisposes for autoimmunity once the incidence of infection is strongly reduced.[114] This is because, while the environmental factors can change very fast, on the contrary the selective pressure needs many generations to be nullified.

Conclusions and perspectives

Genome-wide association studies (GWAS) have uncovered new areas of investigation into the association between different diseases/traits and a large number of genetic loci. Since 2005,[115] GWAS have revolutionized the study of complex traits, yielding to-date more than 24,218 unique SNP-trait associations from 2,518 publications. This somehow implies that, quite frequently, genotype and phenotype are linked, more generally, the mechanisms by which each gene affects the disorders remain largely unknown, and often are not discussed within the reports. It requires the support of functional indications and tools for downstream statistical and bioinformatic analyses.

Toward this aim, the first test to perform after finding a genetic variant associated with a phenotype is to assess the variant activity predictions, using the already mentioned C-score and VEP analysis tool, and others such as PredictSNP2 (https://loschmidt.chemi.muni.cz/predictsnp2/)[116]. They are able to visualize, annotate and prioritize such data to guide the analyst toward a more focussed work hypothesis. However, if they do not make concrete predictions about the role of the polymorphisms, there are many additional databases to consult. For example, if a polymorphism changes the DNA sequence necessary for the binding of a transcription factor (TF), this can be tested by searching in TF databases,[117] but it gives a probabilistic answer that will likely require further tests.

Moreover, a polymorphism does not necessarily regulate the nearest gene. For instance, a systematic study of complex phenotypes and associations in the GWAS Catalog found evidence that affected genes are often up to 2 MB from the associated SNP.[118] To address this issue, a number of techniques have been developed, usually variations on the chromosome capture technique,[119] which allows the isolation and identification of chromosome sequences that interact with one another. While the data are dependent on the tissue in which the experiment was performed, the state of the art is quite advanced now, and a browser has been constructed to allow visualization of this interacting regions information (http://promoter.bx.psu.edu/hi-c/).[120]

Another issue to consider is that the spectrum of RNA products of RNA polymerase II has expanded considerably over the last years. It now includes numerous non-coding RNA products, including some that function as enhancers (enhancer RNAs) and thus influence transcription.[121] Another important milestone that can aid researchers to correlate genomic data with disease and other phenotypic traits, for example by identifying elements responsible for tissue-specific expression, was the ENCODE project (https://www.encodeproject.org/). Because of the great importance of these expression elements, EnhancerAtlas has been assembled.[122] This atlas provides expression information from 105 Human cell lines or tissues, using eight different measures of enhancer activity and DNA accessibility to RNA polymerase II. Consulting these diverse sources may allow more specific hypotheses to be formulated, and ultimately tested. When these hypotheses are validated, they may offer more specific information: cell of interest, enhancer or TF of interest, even revealing non-proximal genes that are actually mediating the phenotype. So, in the end, the route may be longer, but the destination more satisfying, when reached.

Other useful bioinformatic tools, although still in an early phase of verification, are represented by several algorithms developed to test for riboSNitch activity in transcripts and have had some success.[79,84,86]

The lack of an immediate answer when a trait modifying genetic association is detected may first be disheartening, but it is likely just a consequence of the under-appreciated role of non-protein based mechanisms as phenotypic mediators. The spectrum of RNA species and especially RNA polymerase II products is widening.[123] However, it should be kept in mind that as exploration of these different elements and mechanisms, and their cellular roles proceeds, explaining genetic associations with complex traits could provide even more precise indications of disease mechanisms and points of possible intervention than mere amino acid changes. Ultimately, these discoveries will set the stage for more precise and effective interventions.

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Sidebar title: Key concepts

- **Allele:** each form of a gene present in a specific chromosomal position. A genetic variant is characterised by at least two alleles, thus two alternative forms of it.
- **Allelic frequency**: the number of times a specific allele is observed in a population divided by the total number of copies of all the alleles at that particular genetic locus in the same population.
- **Quantitative trait**: is any measurable phenotype, such as height, weight and the level of cells in the blood, which have a continuous distribution. Quantitative traits are generally regulated by several genetic variants and by environmental factors, thus they can be considered as complex traits.
- **Endophenotype**: any quantitative trait with a clear genetic connection with a disease condition.
- **GWAS**: is a genetic study that assesses the association of genetic variants localized in the entire genome with a phenotype. For GWAS, the significance threshold, expressed as the p value of the statistical test used, is generally 5×10^{-8}, which corresponds to the nominal threshold of 0.05 corrected for the number of independent genetic variants assessed (estimated to be at least 10^{6}), thus the higher the number of genetic variants interrogated, the lower the p value threshold.
- **Pleiotropy**: phenomenon by which a gene can influence two or more phenotypes.
- **Expression quantitative trait locus (eQTL)**: is a genetic variant that contributes to the variation of the mRNA level of one or more genes.
- **Long non coding RNA (lncRNA)**: transcript longer than 200 nucleotides that is not translated into protein and can regulate gene transcription and translation.
- **Linkage Disequilibrium (LD):** non random association between alleles at different loci. If two or more alleles at each locus are frequently present together, it can render the task of finding the allele responsible of the association, the “causal” allele, difficult, or impossible.
- **Haplotype block:** a set of closely linked DNA alleles on one chromosome that are often inherited together.
- **RNA folding landscape:** the set of folding conformations that a single RNA transcript may assume.

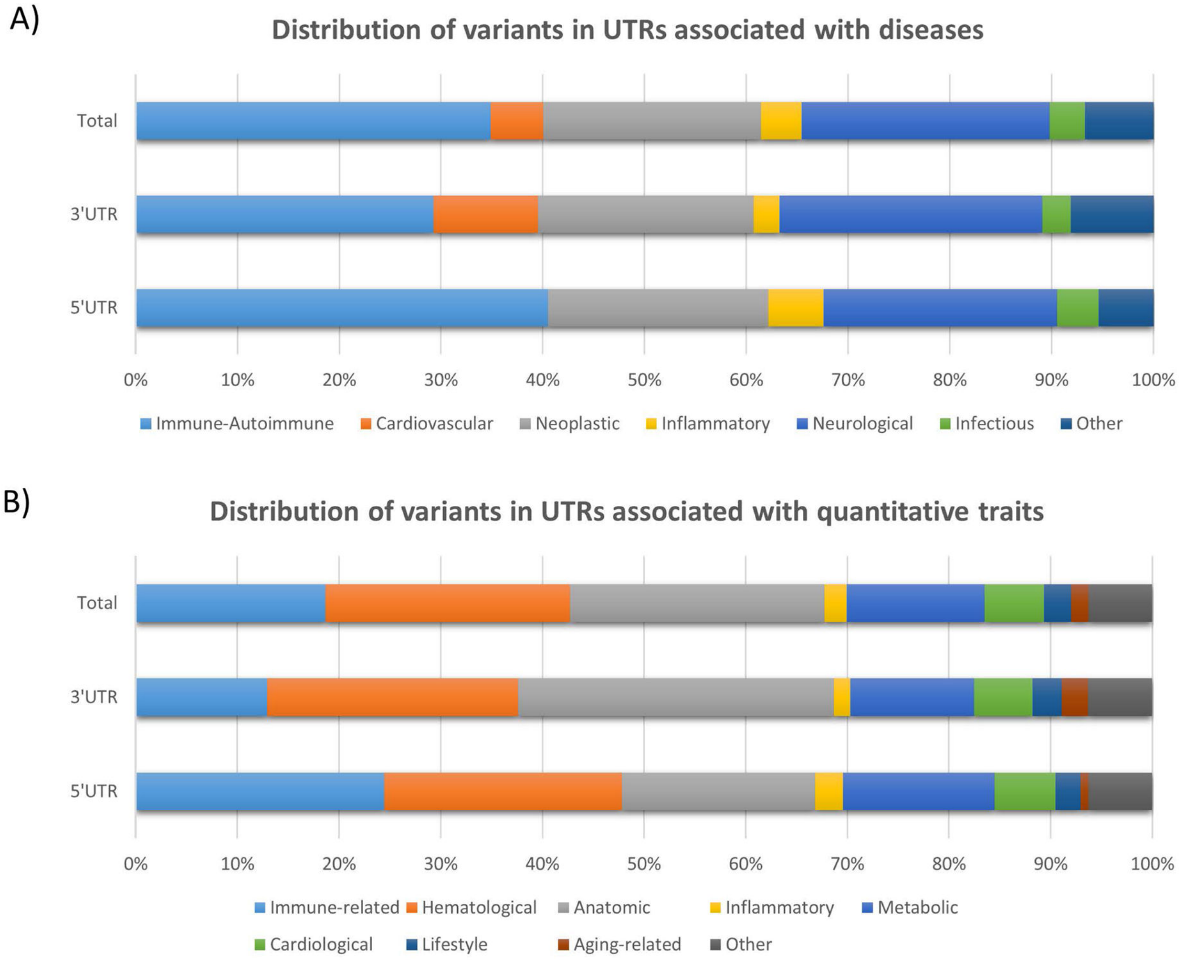

Figure 1. Distribution of UTR variants among associated diseases and quantitative traits

Representation of UTR variants distributed among associated diseases and quantitative traits, as reported in GWAS Catalog. Variants are considered at 5′UTR and 3′UTR jointly (indicated as “total”), and separately. Diseases are categorized in seven non-overlapping classes (panel A), while quantitative traits in nine categories (panel B). In each panel, the percentage of variants associated with phenotypes in the defined categories is reported.

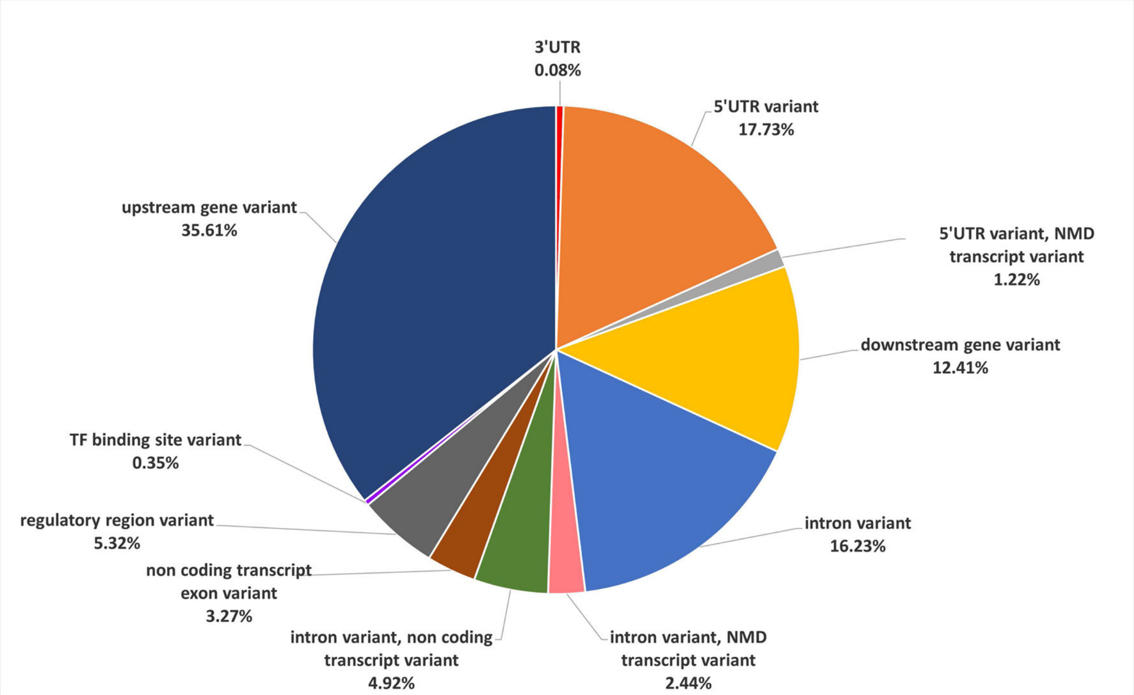

Figure 2. Alternative annotations of 5′UTR variants

The pie graph summarizes the 5′UTR variants reported in the GWAS Catalog and shows their alternative predicted localization due to the presence of gene isoforms.

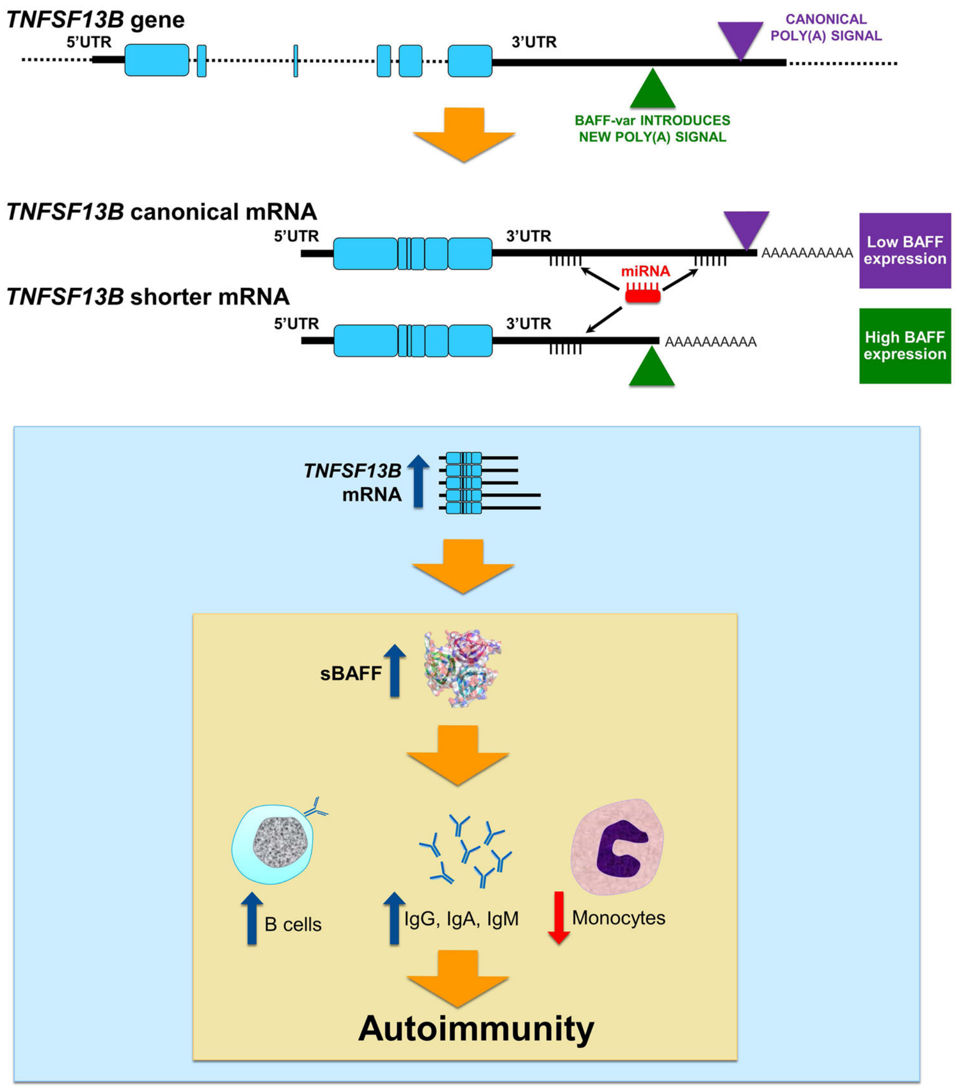

Figure 3. BAFF-var effects at the transcription, protein and cellular level

Representation of the localization of BAFF-var within *TNFSF13B* gene and its effects on the generation of mRNAs with different 3′UTR lengths. The number and location of microRNA sites are reported. BAFF-var creates an alternative polyadenylation signal that generates a shorter 3′UTR transcript lacking a miRNA binding site. In contrast to the wild type allele which produces only with long 3′UTR, BAFF-var leads to a mixed population of mRNAs with long and short 3′UTRs, resulting in higher production of sBAFF. In turn, the increased sBAFF levels lead to higher numbers of B cells and immunoglobulins, reduced levels of monocytes, and increased risk for autoimmunity.

Table 1

Summary of GWAS associations in reported in the UTRs

The table reports, from left to right: the number of variants included in the GWAS Catalog (accession date: 2017-09-19, version 1.0), the significance threshold (p value) considered, the number of variants in the UTRs, and the number of variants in the 5′UTR and 3′UTR. In parentheses are the percentages with respect to variants mapping in the UTRs and percentages with respect to variants present in the entire genome (indicated as "total") are specified.

Total N	Significance threshold	N in UTR	N in 5′UTR (% to the UTR; % to the total)	N in 3′UTR (% to the UTR; % to the total)
57,671	p value<9×10^{-6}	2,094	442 (21.1; 0.8)	1,652 (78.9; 2.9)
	p value<5×10^{-8}	1,362	321 (23.6; 0.6)	1,041 (76.4; 1.8)

Table 2

Genomic distribution of UTRs associations

The table reports, from left to right: the UTR type, the number of variants included in the GWAS Catalog and localised in the UTRs, the average length of the 5′UTR and 3′UTR expressed in bp and, in parentheses, the number of genes used in the calculation, the genomic length calculated as the product of the average length for the number of genes considered, and the number of associated variants per each kb of UTRs' length. The average length has been calculated using all genes associated with an Ensembl Identifier and having an UTR.

Region type	N GWAS variants in the region	Average length (N genes)	Genomic length	N variants per kb
5′UTR	442	151.5 bp (18,714)	2,835 kb	0.16
3′UTR	1,652	872.2 bp (18,770)	16,371 kb	0.10

Table 3

Examples of genetic variants in UTRs reported in the GWAS Catalog and associated with diseases

The table reports, from left to right: the UTR region where each variant is located; the affected disease; the gene where the variant is predicted to map; the disease-associated polymorphism (SNP); the risk allele frequency; its statistical significance, expressed as p value; the impact of the associated variant on disease, expressed as odds ratio; and the Pubmed ID of the paper where the result has been reported, as indicated in GWAS Catalog.

UTR region	Disease	Mapped gene	SNP	Risk allele frequency	p value	Odds ratio	Pubmed ID
5′UTR	Duodenal ulcer	PSCA	rs2294008	0.37	2.E-33	1.84	22387998
	Primary biliary cholangitis	IL21R	rs2189521	0.70	4.E-16	1.41	28425483
	Chronic hepatitis B infection	CD40	rs1883832	0.37	3.E-15	1.19	25802187
3′UTR	Testicular germ cell tumor	KITLG	rs995030	0.80	2.E-50	2.26	23666240
	Venous thromboembolism	F5	rs6427196	0.93	4.E-51	2.07	23650146
	Dementia and core Alzheimer’s disease neuropathologic changes	PVRL2	rs6857	0.29	2.E-62	1.61	25188341
	Chronic hepatitis B infection	HLA-DPB1	rs9277535	0.58	1.E-70	1.52	25802187
	Chronic lymphocytic leukemia	IRF4	rs9391997	0.49	9.E-22	1.35	26956414
	Atrial fibrillation	TBX5	rs883079	0.42	5.E-15	1.18	28416822
	Type 2 diabetes	LOC105375716, SLC30A8	rs3802177	0.70	2.E-18	1.16	24509480
	Coronary heart disease	ZPR1	rs964184	0.13	1.E-17	1.13	21378990
	Epithelial ovarian cancer	HOXD3	rs711830	0.32	3.E-15	1.12	28346442
	Myocardial infarction	CELSR2	rs7528419	0.80	1.E-15	1.11	26343387
	Breast cancer	SLC4A7	rs4973768	0.47	2.E-30	1.10	23535729
	Inflammatory bowel disease	FEN1	rs4246215	0.34	2.E-15	1.08	23128233

Table 4

Examples of genetic variants in UTRs reported in GWAS Catalog and associated with quantitative parameters

The table reports, from left to right: the UTR region where each variant is located, the associated quantitative trait, the gene where the variant is predicted to map, the associated polymorphism (SNP), the frequency of the tested allele, its statistical significance (p value), the impact of the associated variant on the trait, and the Pubmed ID of the paper where the result has been reported, as indicated in GWAS Catalog.

UTR region	Quantitative trait	Mapped gene	SNP	Tested allele frequency	P-value	Effect of the tested allele	PubmedID
5′UTR	HDL cholesterol levels	APOA5	rs651821	0.72	9.E-47	0.19	28334899
	Complement C3 and C4 levels	GTF2H4	rs1052693	0.36	3.E-48	0.10	23028341
	Mean platelet volume	ODF3	rs11604127	0.24	7.E-163	0.12	27863252
	Acylcarnitine levels	SLC16A9	rs1171614	0.20	2.E-81	0.10	26068415
	Platelet count	ODF3	rs11604127	0.24	4.E-103	0.09	27863252
	Height	CNPY2	rs3809128	0.21	7.E-35	0.08	25429064
	Monocyte percentage of white cells	LTBR	rs10849448	0.76	4.E-46	0.06	27863252
	Hip circumference adjusted for BMI	GDF5	rs143384	0.57	1.E-31	0.04	25673412
	Mean corpuscular hemoglobin	TBX6	rs3809627	0.40	5.E-33	0.04	27863252
	Serum ferritin levels	PMS1	rs5742933	0.22	2.E-10	0.11	25162662
3′UTR	Triglycerides	ZPR1	rs964184	0.13	7.E-240	16.95	20686565
	Soluble ICAM-1	ICAM1	rs281437	0.30	3.E-10	10.10	18604267
	Platelet count	SH2B3	rs739496	0.11	7.E-12	8.25	25705162
	Caffeine metabolism (plasma 1,7-dimethylxanthine (paraxanthine) to 1,3,7-trimethylxanthine (caffeine) ratio)	AHR	rs11400459	0.64	5.E-10	6.23	27702941
	LDL cholesterol	CELSR2	rs629301	0.22	1.E-170	5.65	20686565
	Cholesterol, total	CELSR2	rs629301	0.22	6.E-131	5.41	20686565
	Age-related macular degeneration	NELFE	rs522162	0.93	2.E-10	2.33	23577725
	Menarche (age at onset)	TRIM66	rs4929923	0.36	1.E-08	2.30	21102462
	Hypertriglyceridemia	ZPR1	rs964184	0.30	5.E-35	1.77	23505323
	Alcohol consumption (drinkers vs non-drinkers)	OAS3	rs2072134	0.16	3.E-16	1.58	28485404
	Blood protein levels	LEPR	rs17415296	0.19	4.E-229	1.40	28240269
	Allergic sensitization	STAT6	rs1059513	0.90	1.E-14	1.30	23817571
	Obesity	KCNMA1	rs2116830	0.80	3.E-10	1.26	21708048
	Allergic sensitization	IL1RL1	rs3771175	0.86	5.E-11	1.20	23817571

UTR region	Quantitative trait	Mapped gene	SNP	Tested allele frequency	P-value	Effect of the tested allele	PubmedID
	QT interval	LIG3	rs1052536	0.53	6.E-25	0.98	24952745
	End-stage coagulation	MCF2L	rs10665	0.88	2.E-47	0.85	23381943
	Cholesterol, total	HMGCR	rs12916	0.40	5.E-74	0.68	24097068
	Resting heart rate	KIAA1755	rs6123471	0.46	7.E-72	0.60	27798624
	Plasma omega-6 polyunsaturated fatty acid levels (gamma-linolenic acid)	FADS1	rs174546	0.66	5.E-171	0.52	26584805
	Urinary metabolites (H-NMR features)	ACADS	rs3916	0.28	2.E-22	0.40	24586186
	HDL Cholesterol - Triglycerides (HDLC-TG)	APOA5	rs2266788	0.09	5.E-13	0.39	21386085
	Red blood cell fatty acid levels	FADS1	rs174545	0.33	8.E-90	0.37	25500335
	Metabolic syndrome	APOA5	rs2266788	0.09	2.E-09	0.26	21386085
	Metabolite levels (lipoprotein measures)	ZPR1	rs964184	0.86	8.E-66	0.24	27005778
	IgE levels	STAT6	rs1059513	0.11	2.E-12	0.12	22075330
	Cognitive function	TOMM40	rs10119	0.29	6.E-09	0.04	25644384

Table 5

C-score values for UTR-associated variants with diseases and related traits

The table reports, from left to right: the UTR region where each selected variant is located; the rsID of the polymorphism (SNP); the associated quantitative trait or disease; the gene where the variant is predicted to map; the statistical significance of the variant, expressed as p value; its impact on the trait/disease; the C-score; and the Pubmed ID of the paper where the result has been reported, as indicated in GWAS Catalog.

UTR region	SNP	Trait/disease	Mapped gene	p-value	Effect of the tested allele	C-score	PubmedID
5′UTR	rs2236293	Blood protein levels	TMEM8B	5.0E-14	0.33	17.96	28240269
	rs149698681	Granulocyte percentage of myeloid white cells	CAPN3	2.0E-10	0.08	13.83	27863252
3′UTR	rs78378222	Mean corpuscular hemoglobin	TP53	6.0E-09	0.10	17.97	27863252
	rs45474992	Monocyte count	BBC3	3.0E-09	0.06	17.19	27863252
	rs1128334	Systemic lupus erythematosus	ETS1	7.0E-12	1.39	16.20	26663301
	rs2297991	Cholesterol, total	GPAM	8.0E-10	0.04	15.46	25961943
	rs11553699	Reticulocyte fraction of red cells	TMEM120B, RHOF	3.0E-09	0.03	14.74	27863252
	rs6796	Mean platelet volume	KDELR2	5.0E-15	0.03	14.21	27863252
	rs16850073	Monocyte percentage of white cells	CXCL6	5.0E-13	0.03	13.39	27863252
	rs4233366	Asthma	PPOX - ADAMTS4	5.0E-15	1.09	12.63	27182965

Table 6

Summary of results described in this overview

The table reports, from left to right: the affected gene and its *trans* regulator (if any); the UTR region where the studied genetic variant is localized; the associated disease; the reference where the mechanism reported has been described.

Affected gene_*trans* regulator	**UTR region**	**Associated disease**	**Reference**
uORF			
SPINK1	5′UTR	Hereditary pancreatitis	Calvo, 2009
HR	5′UTR	Marie Unna hereditary hypotrichosis	Wen, 2009
HT3A	5′UTR	Bipolar disorder	Niesler, 2001
IRES			
*c-MYC*_ITAFs and YB-1	5′UTR	Multiple myeloma	Cobbold, 2010
connexin-32	5′UTR	Charcot-Marie-Tooth disease	Hudder, 2000
RNA-binding protein			
*TPH2*_unknown	5′UTR	Behavioural traits and psychiatric disorders	Chen GL, 2008
*FMR1*_TDP-43	5′UTR	Fragile X-associated tremor/ataxia syndrome	He, 2014
5′UTR deletion			
ATP7B	5′UTR	Wilson disease	Cullen 2003
CCG repeat			
FMR1	5′UTR	Fragile X syndrome	Yifan Zhou, 2016
Kozak sequence			
β-globin	5′UTR	β-thalassaemia	Angioletti, 2004
3′UTR length and polyadenylation signal			
HTT	3′UTR	Huntington disease	Romo, 2017
FOXP3	3′UTR	Polyendocrinopathy, enteropathy, X-linked	Bennet 2001
α and β globin	3′UTR	Thalassemia	Orkin 1985; Rund 1992; Higgs 1983
TNFSF13B	3′UTR	Multiple sclerosis, Systemic lupus erythematous	Steri, 2017
MicroRNA			
*IZKF3*_miR 326	3′UTR	Autoimmune diseases	Richardson, 2011
*PVRL*_miR 320	3′UTR	Alzheimer Disease	Ghanbari, 2016
*KRAS*_miR let-7a	3′UTR	Metastasis in osteosarcoma	Zhang, 2017

Affected gene_*trans* regulator	**UTR region**	**Associated disease**	**Reference**
*APOA5*_miR-485-5p	3′UTR	Metabolic syndrome	Caussy et al, 2014
*TNFSF13B*_miR 15a	3′UTR	Multiple sclerosis	Steri, 2017
RNA-binding protein			
TNFα_HuR	3′UTR	Host defence	Di Marco, 2001
*PPP1R3*_unknown	3′UTR	Type 2 diabetes	Xia, 1999
*TS*_AUF1	3′UTR	Rheumatoid arthritis and several malignancies	Pullmann, 2006
Alternative translation initiation codon			
PSCA	5′UTR	Duodenal ulcer	Tanikawa, 2012
Unknown mechanism			
APOA5	5′UTR	Metabolic syndrome	Zhou, 2013
ETS1	3′UTR	Systemic lupus erythematosus	Yang, 2010
F5	3′UTR	Venous thromboembolism	Tang et al, 2013
IL21R	5′UTR	Primary biliary cholangitis	Qiu, 2017
KITLG	3′UTR	Testicular germ cell tumor	Ruark, 2013