Functional implications of single nucleotide polymorphisms (SNPs) in protein-coding and non-coding RNA genes in multifactorial diseases

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Abstract

Single nucleotide polymorphisms (SNPs) represent the most common type of variation in the human genome. The SNPs located in protein-coding and non-coding RNA genes are classified as neutral and functional. The neutral have no effect, while the functional affect different biological processes and continually confer risk for multifactorial diseases. Functional SNPs found in the promoters of protein-coding and non-coding RNA genes (microRNAs: miRNAs) termed regulatory SNP (rSNPs) and miRNAs rSNPs (miR-rSNPs), respectively, affect the gene expression. Functional SNPs located on the structure of the precursor mRNAs (exons and introns), mature mRNA (5´ untranslated region [UTR], coding sequence, and 3´ UTR), and primary, precursor, and mature miRNAs are termed structural RNA SNPs (srSNPs) and miR-srSNPs, respectively. The srSNPs affect the splicing (and alternative splicing), srSNPs affect the splicing (and alternative splicing), the translation, stability, amino acid sequence, structure, and function of proteins and interaction between mRNA/miRNAs. Finally, the miR-srSNPs affect the structure, processing and interaction between miRNAs/mRNAs. Functional characterization of potentially harmful risk alleles of the SNPs located in protein-coding and non-coding RNA genes have contributed to an understanding of their functions in the complex diseases. The objective of this review is update the reader on the functional role of the SNPs located in protein-coding and non-coding RNA genes and their relationship with multifactorial diseases.

KEY WORDS: *Single nucleotide polymorphism. Protein-coding gene. Non-coding RNA*

Introduction

Early in the year 2000, two articles were published in two of the journals with the highest impact factor, Nature and Science, and both showed the approximate number of human genome nucleotides and an elevated number of common genetic variants (more than one million); the latter were named single nucleotide polymorphisms (SNPs) or single nucleotide variants (SNVs)[1,2]. The most important characteristics of SNPs are: a) they are located in the entire human genome, at intra-genic and extra-genic regions; b) they represent the most common genetic variants; c) they are generally biallelic; d) they are easily evaluated by automated means; and e) large part of them have direct repercussions on human diseases (Table 1). From the functional point of view, it is vitally important identifying the biological role of the less common alleles of the SNPs, located in different genes[3]. By means of candidate gene or genome-wide association studies (GWAS), genes involved with several complex diseases have been identified (Fig. 1)[4-6]. On the other hand, molecular biology-genetics and biochemical studies have contributed to identify the functional role in vitro or in vivo of the less common alleles of SNPs located in the structure of protein-coding and non-coding genes (Fig. 1)[3,7-10]. These studies have defined the functional role of less frequent

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Table 1. Single-nucleotide polymorphysms characteristics

Characteristics	Description
Distribution	On average, one SNP is found for each 250 bp; nearly 11 million have been reported
Location	Inter-genic (or extra-genic) and intra-genic regions; protein-coding and non-coding genes are found in the latter region.
Allele number	Generally, they are biallelic, although they can be triallelic and quadriallelic
Biological effect	Neutral and functional
SNPs assessment	Easily genotyped by means of automated technologies
Uses in health	Identification of individuals genetically susceptible to develop multifactorial diseases, severity, activity and response to medications

alleles in gene expression, splicing (and alternative splicing), translation, protein and micro-RNA (miRNA) structure and function alteration, messenger RNA (mRNA) stability and miRNA and mRNA interaction. Moreover, these studies have contributed to understand how the less common alleles of SNPs confer risk for the development of human multifactorial diseases and how they affect the response to certain medications[3-10].

SNPs functional classification

Functional SNPs are classified according to the region where they are located in and to the effect they exert

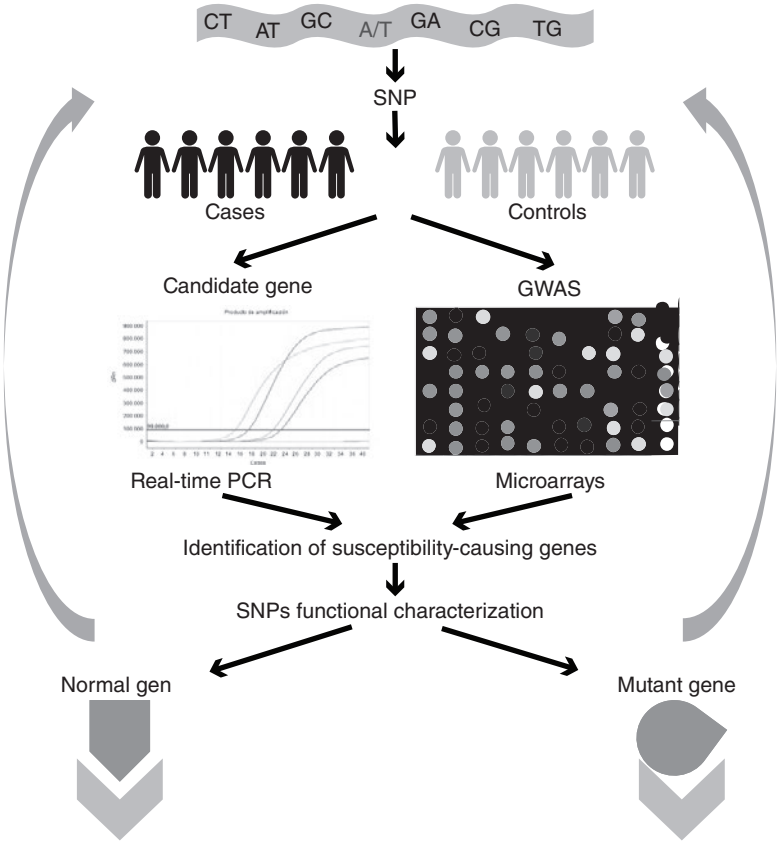

Figure 1. *SNPs functional characterization. Candidate gene or genome-wide association studies (GWAS) have enormously contributed to identify loci involved with risk for the development of different multifactorial human diseases. In turn, molecular genetics and biochemical studies have contributed to identify the functional effect of the less common alleles of the SNPs. These studies can range from candidate gene to functional identification or vice versa.*

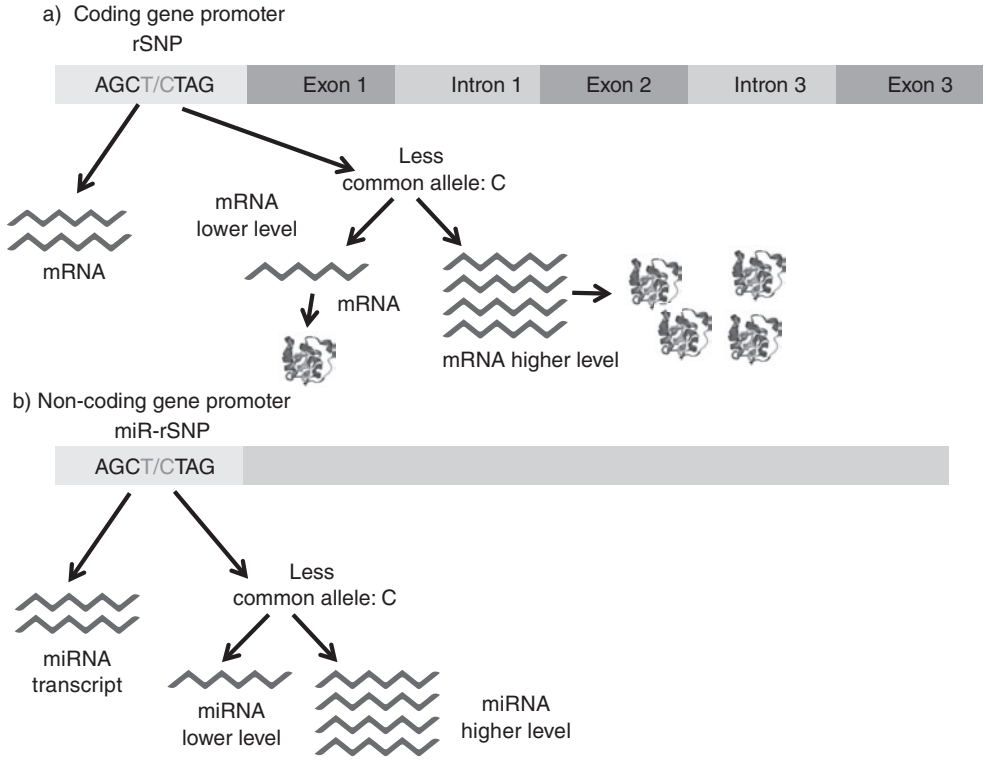

Figure 2. *rSNPs and miR-rSNPs functional effect. rSNPs and miR-rSNPs less common alleles, located in protein coding and non-coding gene promoters, respectively, lead to lower or higher gene expression, either by creating, destroying or modifying the binding affinity of different proteins, such as transcription factors.*

on it. Functional SNPs located in protein-coding and non-coding genes are named regulatory SNPs (rSNPs) and microRNA regulatory SNPs (miR-rSNP), respectively (Table 2); both variants affect gene expression (Fig. 2)[3,11,12]. On the other hand, functional SNPs located in precursor mRNA structure (pre-mRNA) and mature mRNA are named structural RNA SNPs (srSNPs), whereas microRNA srSNPs are known as miR-srSNPs. srSNPs affect mRNA translation, splicing (and alternative splicing), structure and stability, protein maturation, function, and binding between miRNA and mRNA (Fig. 3 and Table 2)[3,11]. In turn, miR-srSNPs affect splicing, large (primary miRNA: pri-miRNA) and precursor (pre-miRNA) transcript processing, miRNA and mRNA binding and their function (Fig. 4). Functional SNPs of the coding sequence are known as synonym (sSNP) and non-synonym (nsSNP) SNPs (Fig. 3). sSNPs involve a nucleotide and codon change (degenerate gene code: more than one codon originates the same amino acid), but not an amino acid change, and although they do not change the amino acid sequence in proteins, they can affect certain traits (Table 2 and Fig. 3). On the other hand, nsSNPs are subdivided in nonsense and missense nsSNPs; the former generate a stop codon and protein premature termination, and the latter generate an amino acid change. Both can have a drastic effect, but with the latter it may not be serious if the replaced amino acids are similar in chemical structure and biochemical properties. Both variant types affect the protein sequence, structure and function (Table 2 and Fig. 3)[3,13-16].

Protein-coding and non-coding gene promoters structure and function

The protein-coding and non-coding gene promoter coordinately regulates gene expression. Different sequences that act at *cis* are found in this region. In the coding gene promoters, the *cis* sequences are found in the basal promoter or core promoter and in the region next to the basal promoter[3]. On the other hand, non-coding gene promoters' consensus sequence, structure and organization are poorly known in comparison with coding gene promoters[17]. Some regulatory elements found in the promoter of both types of genes are the TATA box, elements that recognize general transcription factor II B, the initiating sequence and more than half miRNA promoters are

Table 2. SNPs functional classification

Functional SNPs	Location	Function
rSNPs and miR-rSNPs	Protein-coding and non-coding genes promoter, respectively	Alter gene expression
– srSNPs	pre-mRNA and mature mRNA	Alter mRNA/miRNA translation, stability, length and interaction
– miR-srSNPs	pri-, pre- and mature miRNA	Affect miRNA structure, processing and function
cSNPs	Coding sequence	Affect protein or enzyme structure and function or activity
– sSNPs		
– nsSNPs		
• Nonsense		Nonsense nsSNPs generate a stop codon and protein premature termination
• Missense		Missense nsSNPs generate amino acid changes

cSNPs: coding SNPs; miR-rSNPs: microRNA regulating SNPs; miR-srSNPs: microRNA RNA structural SNPs; nsSNPs: non-synonym SNPs; rSNPs: regulatory SNPs; srSNPs: structural RNA SNPs; sSNPs: synonym SNPs.

associated with CpG islands[17]. Different *cis* sequences located at this region regulate the binding of different transcription factors, transactivators, etc., which act in *trans*. The *cis-trans* bond coordinately regulates gene expression.

rSNPs and miR-rSNPs and biological implications in complex diseases

A large proportion of rSNPs have been identified in the human genome (35,000-47,000); however, the

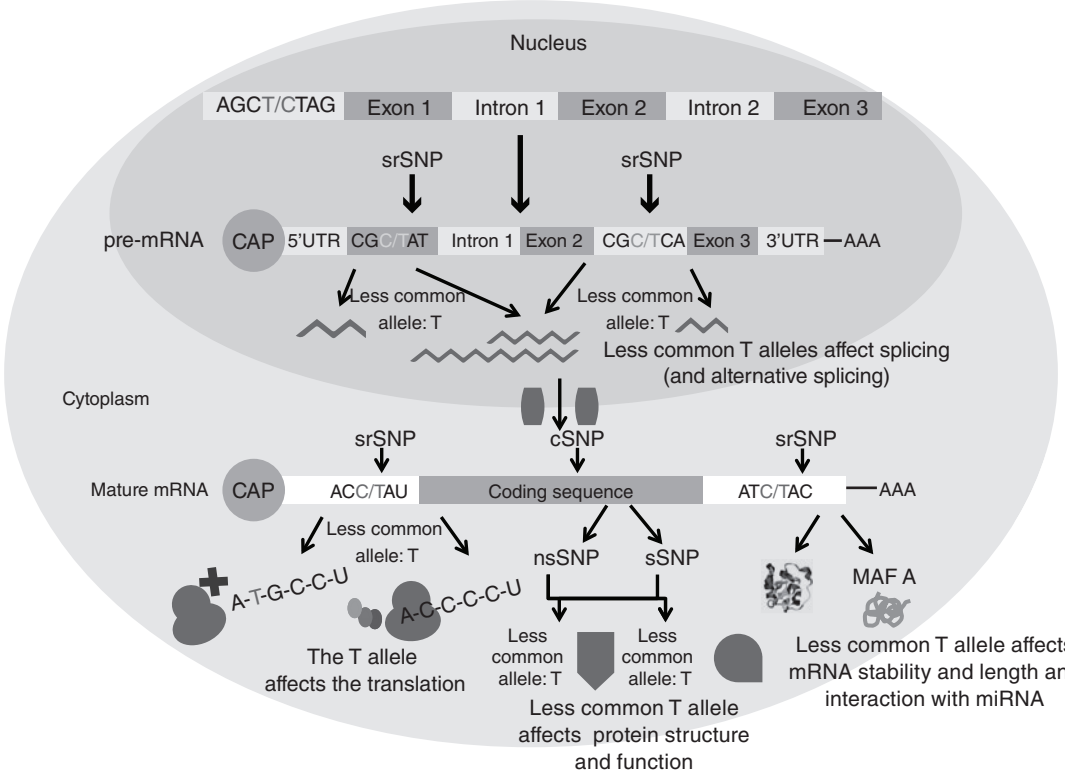

Figure 3. *Functional effect of the srSNPs located in the pre-mRNAs and mature mRNAs. The less common alleles found in introns and exons of pre-mRNAs affect splicing (alternative splicing and efficiency). In turn, srSNPs located in mature mRNA affect the translation, stability, half life and the interaction mRNA/miRNAs; in addition, they can affect the coding sequence, structure and function of proteins.*

number of miR-rSNPs located in non-coding genes has not been reported[18,19]. The C allele of the *FCRL3* -169T/C SNP, which encodes for Fc receptor like 3 protein, increases FCRL3 expression by modifying the binding affinity for transcription factor NF-κB, and confers susceptibility for the development of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)[20]. On the other hand, rSNPs -376G/A, -308G/A and -238G/A of the *TNF*-α gene, which encodes tumor necrosis factor alpha, lead to increased mRNA and protein expression, and increase the risk for the development of chronic inflammatory and autoimmune diseases[3,21]. Individuals with GA and AA genotypes of *TNF*-α -308G/A rSNP fail to respond to anti-TNFα biologic therapy in comparison with the common genotype, G/G[3,21]. In turn, *MHC2TA* -168G/A rSNP, which encodes major histocompatibility complex class II transactivator, reduces its expression in vitro and confers risk to develop RA, multiple sclerosis and acute myocardial infarction[22]. Another example is the –125G/A rSNP of *BAX* gene, which encodes pro-apoptotic protein BAX (a tumor-suppressor gene): the A allele leads to lower mRNA-protein levels expression in comparison with G allele, and confers risk for the development of chronic lymphoblastic leukemia[23]. Other rSNPs located in other genes alter the degree of gene expression and confer susceptibility for the development of complex human diseases[24-26]. On the other hand, few miR-rSNPs have been reported to affect gene expression. The G allele of the *miR-146a* rs57095329A/G miR-rSNP leads to lower expression of miR-146a, which negatively regulates several genes of the interferon pathway (a marker of severity and activity in SLE), and miR-146 decrease confers risk to develop SLE[27]. the C allele (found at CpG island) of the *miR34b/c* rs4938723C/T miR-rSNP decreases its expression in vivo and in renal tumor cells and increases the risk for the development of this tumor[28]. Other miR-rSNPs located in different miRNA genes affect their levels of expression and confer susceptibility for the development of several human diseases[29-31].

mRNA structure

Given the importance of each mRNA region, their structure and function will be briefly described. Different regions form precursor (pre-mRNA) and mature mRNA, and each region participates in different biological events. pre-mRNAs are comprised by:

- Exons and introns, and at 5' and 3' ends of the transcript, the cap structure and poly-adenine (polyA) tail, respectively, are found.
- In turn, mature mRNA, or simply mRNA, is formed by untranslated region (UTR) 5'.
- Coding sequence.
- 3' UTR, and at 5' and 3' ends of the transcript, the cap and polyA, respectively, are found; introns have already been eliminated (Fig. 3).

5' UTRs structure and function

At the 5' end of the 5´UTR of primary and mature mRNA the cap is found, and on this region there are several *cis* sequences that regulate translation; these include the internal ribosome entry site (IRES), the translation start codon (AUG) and other alternative upstream-located AUGs. The presence of more than one IRES, AUG and different secondary structures (e.g., stem-loop) importantly regulates mRNA translation. In this region, different proteins are bound, which regulate mRNA entry to ribosomes, translation initiation-elongation and, frequently, gene expression (due to closeness of this region to the gene promoter). In eukaryotes, translation initiation depends on two events: cap-dependent initiation and mRNA entry to ribosomes[32-34]. Translation initiation starts when the IF4E factor binds to cap, immediately after, eIF4F is bound and a complex is formed that consists of eIF4E, eIF4A and eIF4G. eIF4A, an ATP-dependent RNA helicase, unfolds 5' UTR region secondary structure stimulated by RNA-binding protein: eIF4B. Subsequently, mRNA binds to ribosomal small subunit 40S; this subunit finds AUG and initiates protein synthesis[32-34].

srSNPs located in the 5' UTR of protein-coding genes and their biological implications in complex diseases

Different srSNPs located in the 5' UTR have been characterized at the functional level. The +112A/C srSNP located in exon 1 of *UCP1*, which encodes uncoupling protein 1, is located in an element of response for insulin. One study showed that the less frequent allele alters mRNA levels and confers risk for type 2 diabetes mellitus[25]. srSNP rs3813946A/G of *CR2*, which encodes complement receptor 2, not only affects its gene expression, but also alters accessibility of some proteins to chromatin and confers susceptibility for the development of SLE[36]. On the other hand, srSNP rs751404C/T of *ERCC5* gene, encodes an endonuclease (excision repair 5, endonuclease) and is involved in nucleotide excision repair, creates and open reading frame upstream of the original, and affects the

expression of the protein and its capability to be synthesized after damage to DNA. Individuals with the risk variant show resistance to platinum-based drugs[37]. Other srSNPs located at this region affect mRNA translation or the degree of expression of their respective genes and confer susceptibility for the development of multifactorial human diseases, such as asthma, insulin resistance and psychiatric disorders[38-40].

Introns structure and function

Introns are non-encoding sequences located between protein-coding gene exons or pseudogenes. Generally, they are large when compared with exons, and can be hundreds to thousands-nucleotide long. A recent study showed that these sequences account for nearly 24% of the entire human genome[41-43]. Splicing is the biological mechanism by means of which introns are eliminated and exons are bound. Recent data indicate that 70%-98% of protein coding genes undergo this process[3]. These sequences were initially proposed to be junk DNA (since they are eliminated from pre.RNA), but now we know that they contain miRNA genes (alone or grouped)[44] and several *cis* sequences involved in splicing (and alternative splicing), and in splicing efficiency, such as branching and intronic splicing enhancer (ISE) or intronic splicing silencer (ISS) sites, among others. The spliceosome, which regulates intron elimination and exon binding, is constituted of different small nuclear ribonucleoproteins (snRNP), such as U1, U2, U4, U5 and U6, and non-snRNP related factors[45,46]. A typical intron contains one end 5' splice site (5 ss), one branch point sequence (BPS), one polypyrimidine tract (PYT) and another ss at intron 3' end (Fig. 3), in addition to usually containing ISE or ISS[3,45,47].

srSNPs located in introns and biological importance in complex diseases

Intron-located srSNPs affect splicing, alternative splicing and splicing efficiency. srSNP rs9930761T/C, which changes one thymine for one cytosine, located at intron 8 of the *CEPT* gene, which encodes a protein that transfers cholesterol, is located at the branching site, affects exon 9 inclusion/exclusion, is associated with low-density lipoprotein (LDL) altered levels and confers gender-dependent cardiovascular risk[48]. On the other hand, three srSNPs (rs17026688C/T, rs17026651C/G and IVS8+48delG; the latter one G nucleotide deletion) located at intron 8 of the *GADL1* gene, which encodes glutamate decarboxylase-like protein 1, strongly affect lithium-based therapy in patients with bipolar disorder I. The IVS8+48delG variant affects the 7-8 exons splicing of *GADL1*, thus generating a short isoform due to both exons elimination in a neural cell line, and this event affects the response to lithium (a drug used as first line of treatment in bipolar disorder)[49-51]. On the other hand, the rs2283265T/G and rs1076560T/G srSNPs, located at introns 5 and 6, respectively, of *DRD2* gene that encodes dopamine receptor D2, regulate exon 6 exclusion, which generates a short transcript. Studies carried out in prefrontal cortex and striate tissue confirmed that carriers of GG haplotype (originated of both srSNPs) generate a *DRD2* short transcript that affects SRP55 and SRP40 proteins binding, which regulate splicing and alter neuronal activity and memory[52]. Another example is represented by rs9406328A/G srSNP, located in intron 10 of the *THBS2* gene, which encodes thrombospondin 2, an extracellular matrix protein that regulates the levels of metalloproteinases involved in its remodeling. rs9406328A/G srSNP is found in the polypyrimidine tract. One study showed that this variant affects exon 11 in vivo and confers risk for the development of lumbar disc herniation[53]. Other srSNPs located in different genes' introns affect splicing (and alternative splicing) and splicing efficiency and confer risk for the development of different multifactorial human diseases[54-57].

pre-mRNAs structure and function

pre-mRNAs (with the cap and polyA tail on their 5' and 3' ends, respectively), are formed by exons and introns. Exons form the 5' UTR regions, the coding sequence and 3' UTR; in turn, introns interrupt the coding sequence. pre-mRNAs-located exons play an essential role in splicing (and alternative splicing) regulation, in different proteins binding to mRNAs, in the accessibility of proteins that regulate pre-mRNAs splicing, and in the formation of pre-mRNAs structure and in their stability (Fig. 3). Several *cis* structures have been identified at exons' initiation and end, including exonic splicing enhancers (ESE) and exonic splicing silencers (ESS), which are sequences that respond to splicing activation, and splicing acceptor sites, among others[3,58-60]. These sequences are recognized by snRNP and other proteins, which enhance or inhibit splicing (or alternative splicing) efficiency, generating different mRNAs isoforms, with exons inclusion or exclusion, introns retention, etc., which translates into proteins with different length and acitivity[3,58-60].

srSNPS located at exons of pre-mRNAs and biological importance in complex diseases

srSNPs located at exons of pre-mRNAs affect splicing, alternative splicing and splicing efficacy. C77G srSNP, located at exon 4 of the *CD45* gene, which encodes a protein tyrosine phosphatase type C receptor, is found in one of pyrimidine tract residues. This polymorphism alters ESS1 normal function, inhibits splicing and affects exon 4 inclusion in the CD45 transcript. Elimination of this exon leads to a decrease of its activity in T cells and contributes to immune system hyperactivity and to susceptibility for the development of different autoimmune diseases[59]. On the other hand, srSNP rs5883C/T, located at exon 9 of the *CETP* gene, affects the sequence of an ESE and, together with intron 8-located rs9930761T/C (which affects the branching site), alters exon 9 inclusion and ultimately affects LDL values and cardiovascular risk[48]. In turn, the rs767455A/G rsSNP, located at ss of exon 1 3' end of *TNFR1* pre-mRNA, which encodes tumor necrosis factor receptor 1, affects exon 2 inclusion; this variant has been associated with TNFR-associated periodic syndrome[61]. Other srSNPs affect splicing, splicing efficacy, pre-mRNA structure stability and accessibility of several proteins that regulate splicing, in addition to conferring risk for the development of different complex human diseases (Table 3)[62-64].

Mature mRNA coding sequence structure and function

Mature mRNA coding sequence is involved in the synthesis of different proteins. In this process, the genetic code uses 61 codons (degenerate genetic code) to place 20 different amino acids in proteins. Since several years ago, the coding sequence has been known not only to encode amino acids and synthesize proteins, but to fulfill other functions, such as maintaining mRNA secondary or tertiary structure stable through binding of different RNA-binding proteins (RBPs) that are involved in its folding and that also protect mRNA from degradation[65,66].

sSNPs and nsSNPs in the coding sequence and biological relevance in complex diseases

Theoretically, sSNPs have no effect on cells, but this is not accurate, since pre-mRNA and mature mRNA-located sSNPs can affect these RNAs splicing, structure, folding and stability, the response to medications and interaction with different RBPs (Fig. 3)[13,65-69]. On the other hand, nonsense and missense nsSNPs can affect different proteins' (or enzymes) structure, folding, stability, interaction with other proteins, function and activity, as well as the response to medications[15,69-72]. One sSNP located at exon 26 of the *MDR1* gene, which encodes permeability glycoprotein p (P-gp), works as an efflux pump that contributes to the pharmacokinetics of several medications; it is found in sSNP 3435C/T (isoleucine/isoleucine), and does not alter mRNA and protein levels (levels are similar when both alleles are compared); however, change of the common ATC for the rare ATT codon, affects translation velocity, folding and P-gp insertion to the cell membrane, and leads lower effectiveness to respond to certain medications[73,74]. On the other hand, sSNP 971C/T, located in gene *CDSN*, which encodes corneodesmosine, affects mRNA structure stability (because this variant contains an mRNA stability motif), alters the binding of a protein to mRNA and confers risk for psoriasis (Table 3)[75].

On the other hand, several nsSNPs located in *DNase 1* and *DNase1L3* coding sequence, which encode deoxynuclease 1 and DNase 1-like protein 3, respectively, decrease their activity and confer susceptibility to SLE, since both DNases do not eliminate DNA residues (from nucleosomes), with the possibility for antibodies against DNA to be generated[76]. On the other hand, rs5744174C/T nsSNP, which changes leucine for phenylalanine in the *TLR5* gene, which encodes toll-like receptor 5, increases the production of chemokine 20 in response to flagellin and confers risk for the development of Crohn's disease[77]. The T allele of C1858T nsSNP of the *PTPN22*, which encodes protein LYP, a T cell receptor (TCR)-mediated T/B lymphocyte signaling negative regulator, changes an arginine for a tryptophan in codon 620 (R620W) and ruptures the interaction with C-Src Kinase (CSK) protein; both (with LYP) negatively regulate lymphocyte B/T activation, thus leading to higher T/C cell activation, thus and higher T/B cell activation, thus conferring susceptibility to RA[78-80]. Other sSNPs and nsSNPs affect interaction with other proteins, enzymatic activity, etc., and confer susceptibility to different human diseases (Table 3)[15,65,69,81].

Mature mRNA 3' UTR region structure and function

The presence of *cis* sequences and various structures located in mature mRNA 3' UTR region regulate

Table 3. Example of functional SNPs involved with risk for the development of different multifactorial human diseases

Type of SNP	Gene	Gene position	Molecular alteration and involved pathology(ref.)
rSNP	*FCRL3*	-169T/C	Affects gene expression and confers susceptibility to SLE and RA[20]
rSNP	*TNF-α*	-376G/A -308G/A -238G/A	Affects gene expression and confers risk for cerebral malaria, RA and SLE[21]
miR-rSNP	*miR-146a*	Promoter	Affects gene expression and confers risk for SLE[27]
miR-rSNP	*miR-34b/c*	Promoter	Affects gene expression and confers risk for renal cancer[28]
srSNP	*CR2*	5' UTR	Alters gene expression and accessibility for proteins that regulate expression, confers risk for SLE[36]
srSNP	*ERCC5*	5' UTR	Creates an additional reading frame, affects expression and synthesis of the protein, causes resistance to platinum-based agents[37]
srSNP	*CETP*	Intron 8	Causes splicing alteration and is associated with cardiovascular diseases[48]
srSNP	*GADL1*	Intron 8	Affects alternative splicing and alters the response to lithium-based therapy in bipolar disorder[49]
srSNP	*SLC6A4*	3' UTR	Affects mRNA expression and stability and is associated with higher craving for alcohol[89]
srSNP	*TNFR2*	3' UTR	Alters mRNA stability and is associated with obesity and insulin resistance[90]
srSNP	*miR-146a*	pre-miR-146a G/C stem	Affects miRNA structure stability and processing, and confers susceptibility to prostate cancer, hepatocellular carcinoma and sepsis[100]
srSNP	*miR-196a-2*	pre-miR-196a-2 C/T stem	Affects miRNA processing and confers risk for colorectal cancer[101]
sSNP	*MDR1*	3435C/T exon 26	Affects folding velocity and P-gp function on cell membrane[73]
nsSNP	*TLR5*	Exon	Induces higher amounts of CCL20 in response to flagellin and confers risk for Crohn's disease[77]
nsSNP	*PTPN22*	Exon 14	Alters Csk-LYP bond and is no longer able to inactivate immune system cells, which generates risk for RA[78-80]

CCL20: Chemokine C-C Ligand 20; CETP: Cholesteryl Ester Transfer Protein; CR2: Complement receptor 2; ERCC5: Excision Repair Cross-Complementation group 5; FCRL3: Fc Receptor-like protein 3; GADL1: Glutamate decarboxylase-like 1; MDR1: Multidrug Resistance protein 1; miR-146a: microRNA 146a; miR-196a-2: microRNA 196a-2; miR-34b/c: microRNA 34b/c; miR-rSNP: microRNA-regulator SNP; nsSNP: non-synonym SNP; PTPN22: Tyrosine-protein phosphatase non-receptor type 22; RA: rheumatoid arthritis; rSNP: regulator SNP; SLC6A4: Solute Carrier family 6; SLE: systemic lupus erythematosus; srSNP: structural RNA SNP; sSNP: synonym SNP; TLR5: Toll-Like Receptor 5; TNFR2: Tumor Necrosis Factor Receptor 2; TNF-α: Tumor Necrosis Factor Alpha.

post-transcriptional gene expression, largely owing to interaction with *trans* factors, including BP and miRNAs. This region is involved in mRNAs stability, location and exportation to the cytoplasm, and in mRNAs-miRNAs interaction[82]. 3' UTR length considerably varies and depends on the tissue, organ or pathologic condition. For example, a mRNA that encodes the same protein can have a 3' UTR with variable length in different cell types owing to alternative polyadenylation sites contained by this region[83,84]. 3' UTR short isoforms are associated with higher stability, whereas the long ones are associated with lower stability, and short isoforms escape mechanisms that regulate mRNA degradation owing to bonds with different miRNAs, which suppress mRNA translation; this way, long 3' UTRs display a larger number of miRNA binding sites. Other sequences located at this region regulate mRNA half life decline[83-87].

3' UTR region-located srSNPs and biological significance in complex diseases

The rs3735590C/T srSNP of the *PON1* gene, which encodes paraoxonase 1 protein, affects miR-616 binding, C allele, reduces miR-616 binding affinity and increases *PON1* gene expression. This variant confers risk for the development of ischemic stroke and subclinical atherosclerosis traits[88]. On the other hand, allele G of sSNP rs1042173T/G, located close to a polyadenylation site and to miR-135 binding site in the 3' UTR region of the *SLC6A4* gene, which encodes

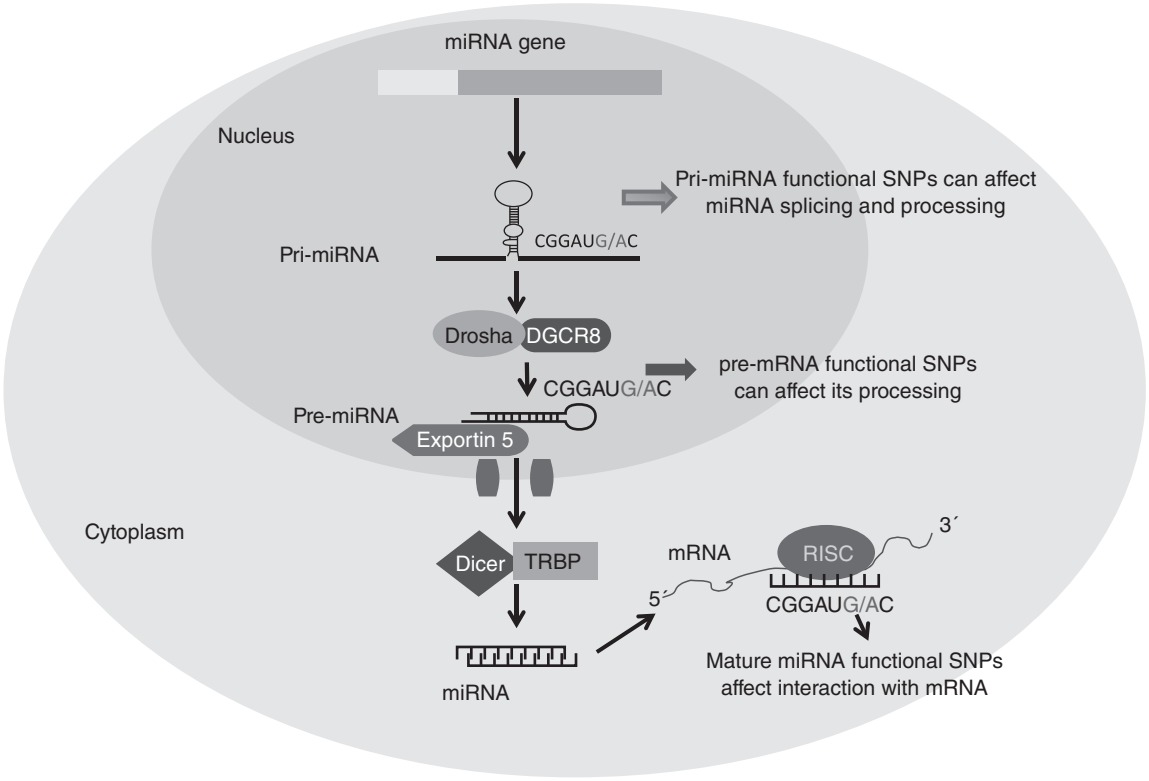

Figure 4. *Functional effect of miR-srSNPs located at pri-miRNA, pre-miRNA and mature miRNA. The less common alleles of SNPs located in pri-miRNAs, pre-miRNAs and mature miRNAs affect splicing (when several miRNAs originate from a single transcript), processing, structure, miRNA and mRNA interaction and, finally, their function.*

serotonin transporter (this gene is expressed in human brain), increases mRNA and protein levels in comparison with T allele. In addition, this variant showed an association with craving for alcohol. Some authors have hypothesized that this variant can affect mRNA stability[89]. Three srSNPs located at the 3' UTR region of the *TNFR2* gene, which encodes tumor necrosis factor receptor 2, affect protein binding to mRNA, half life and stability. In addition, these variants have shown an association with several complex human diseases, such as obesity, leptin increase and insulin resistance, among others (Table 3)[90]. Other variants that affect mRNA stability and binding with different miRNAs and confer susceptibility to different human diseases have been described by several authors (Table 3)[91-93].

Structure and function of genes that produce non-coding RNA. miRNA example

Data on human genome sequencing showed that only 1.5% encodes proteins; the rest of transcripts originate in non-coding genes and includes miRNAs, tRNAs, long non-coding, and rRNAs, among others[94-97]. This section will address specifically miRNA structure and function. *miRNA* genes are distributed in practically all chromosomes; only a couple have been identified in the Y-chromosome. Given their wide distribution and size, they can be identified in intergenic regions, in introns and in exons[44,98]. Most pri-miRNAs are synthesized by RNA polymerase II, and similarly to mRNA, they contain the cap and polyA tail on their 5' and 3' ends, respectively[99]. Mature miRNAs are 18-22-nucleotide long and originate in pre-miRNA of approximately 70 nucleotides, which are generated from larger pri-miRNAs[97]. pri-miRNAs are cleaved by the Drosha RNase, generating pre-miRNAs in the nucleus, which bind to exportin 5 and travel to the cytoplasm, where RNase Dicer III generates 22-nucleotide duplex mature mi-RNAs. Subsequently, an argonaute protein selects the guide strand, while the anti-guide strand is degraded by the RNA-induced silencing complex (RISC), and final product is miRNA, which is ready to bind to its different mRNAs (Fig. 4)[97-99]. Different studies have shown that miRNAs seed region (nucleotide 2-7) is determinant in mRNAs selection[97-99]. miRNAs binds

especially to mRNAs 3' UTR region, and its primary function is to inhibit gene expression at the post-transcriptional level, thus inhibiting protein synthesis[99] (Fig. 4).

3' UTR region-located srSNPs and clinical relevance in complex diseases

Genetic variants in the structure of pri-, pre- or mature miRNA affect its processing, activity and function (Fig. 4). Functional rs2910164G/C miR-srSNP located in *pre-miR-146a* stem region, results in a change of C:U in place of G:U, which affects miR-146a integrity, processing and mature form. In addition, this variant has been associated with different human diseases, such as prostate cancer, hepatocellular carcinoma and severe sepsis, among others[100]. Another functional SNP located at *pre-miR-196a-2* stem region is rs11614913T/C. C Allele of this SNP affects its structure, and functional studies showed that C allele increases *miR-192a-2* levels when compared with T allele in lung tumor tissue. Less common C allele was associated with an increase in mature miR-196a-2 levels. These results indicate that rs11614913T/C miR-srSNP affects pre-miRNA processing into its mature form. Previous studies have shown that high levels of miR-196a promote colorectal cancer cells migration and invasion[101]. On the other hand, a *pre-miR-34a*-located functional SNP reduces its expression and promotes osteosarcoma cells migration and proliferation[102]. Other studies have reported that this type of SNP alters levels of expression, processing or activity, thus conferring susceptibility to develop different multifactorial human diseases (Table 3)[103,104].

Conclusions

Several studies have shown the functional importance of rSNPs, miR-rSNPs, srSNPs and miR-srSNPs located in protein-coding and non-coding genes on the pathophysiology of different multifactorial diseases, since they affect mRNAs and miRNAs gene expression, splicing, stability (and structure) and processing, as well as miRNA/mRNA interaction. In turn, sSNPs and nsSNPs affect the structure, function or activity of proteins or enzymes implied in the response to medications. Understanding SNP alleles biological effect on different genes associated with different diseases will enable to correctly define their influence on susceptibility, severity and activity of different multifactorial human diseases and, in addition, it will contribute to identify those individuals who will respond or not to certain drugs or biological therapies.

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Conflict of interests

The authors declare no conflicts of interest with regard to this review.

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