Published in final edited form as:

Curr Opin Lipidol. 2019 June ; 30(3): 157–164. doi:10.1097/MOL.0000000000000605.

Genetics-Driven Discovery of Novel Regulators of Lipid Metabolism

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Abstract

Purpose of review: Residual cardiovascular disease risk and increasing metabolic syndrome risk underscores a need for novel therapeutics targeting lipid metabolism in humans. Unbiased human genetic screens have proven powerful in identifying novel genomic loci, and this review discusses recent developments in such discovery.

Recent findings: Recent human genome-wide association studies have been completed in incredibly large, detailed cohorts, allowing for the identification of >300 genomic loci that participate in regulation of plasma lipid metabolism. However, the discovery of these loci has greatly outpaced the elucidation of the underlying functional mechanisms. The identification of novel roles for long noncoding RNAs such as *CHROME*, *LeXis*, and *MeXis*, in lipid metabolism suggests that noncoding RNAs should be included in functional translation of GWAS loci.

Summary: Unbiased genetic studies appear to have unearthed a great deal of novel biology with respect to lipid metabolism, yet translation of these findings into actionable mechanisms has been slow. Increased focus on the translation, rather than the discovery, of these loci, with new attention paid to lncRNAs, can help spur the development of novel therapeutics targeting lipid metabolism.

Keywords

Genetics; Lipid Metabolism; GWAS; Coronary Artery Disease; lncRNA

Introduction:

Metabolic disease, including cardiovascular disease among others, has seen a rise in recent years [1], prompting increased interest in understanding the complex biologic processes involved in disease development with the purpose of developing therapeutics. Given the importance of lipids in metabolic health and pathophysiology, a large focus has been placed on understanding the biology of lipid metabolism. Indeed, dyslipidemia, particularly increased circulating total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG), and decreased high-density lipoprotein cholesterol (HDL-C), is a

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Conflicts of interest:

None.

major determinant of cardiovascular risk [2], while accumulation of lipids in tissues such as adipose and liver also have implications in metabolic disease such as in insulin resistance and other obesity-related disorders [3]. Studies have shown that metabolic traits, including plasma lipids, have a large hereditary component as well [4], and understanding the genetic determinants can identify novel biology governing lipids and disease. Human genetic studies have thus accelerated the discovery of genes and pathways that regulate disease as well as potential therapeutic targets. In this review, we discuss the latest advances in discovery of genetic loci associated with plasma lipids, the functional validation of some of these loci that has been performed to date, and recent advances in our understanding of the role of long noncoding RNAs (lncRNAs) in plasma lipid regulation.

Recent genetic studies provide opportunities for discovery of novel genes regulating plasma lipids:

Genome-wide association studies (GWAS), which determine associations between single nucleotide polymorphisms (SNPs) and disease traits, have become an important tool for identifying genomic loci with potential roles in the regulation of disease pathophysiology. Plasma lipids, as major determinants of cardiometabolic risk that are relatively easy to assess in the clinic, have become a major focus of genetic discovery. GWAS for serum triglycerides and cholesterol began to be utilized ten years ago in smaller cohorts (<10,000 subjects) [5–7], leading to the discovery of a few (<30) significantly-associated loci, many of which were already understood to have lipid regulatory roles (including *CETP*, *PCSK9*, *APOB*, and *LDLR*). A decade later, meta-analyses that combine several study cohorts have proven to be a powerful method for identifying large numbers of variants that associate with serum lipids. The Global Lipids Genetics Consortium (GLGC), in particular, has contributed some of the most comprehensive GWAS meta-analyses to the field. In 2010, the GLGC performed a meta-analysis of 46 GWAS, comprising a total of >100,000 European individuals, for plasma lipids and identified 95 genomic loci that were genome-wide significant ($P < 5 \times 10^{-8}$) for one or more plasma lipid traits (TG, LDL-C, HDL-C, and TC) [8]. The GLGC further expanded this list in 2013 to 157 loci through meta-analysis of 60 studies, comprising a total of 188,577 European individuals [9].

A more recent GWAS was conducted by Hoffmann et. al. [10] using data from ~100,000 genotyped Kaiser Permanente members from the Genetic Epidemiology Resource on Adult Health and Aging (GERA) cohort. Remarkably, this was a large-scale GWAS conducted in a single study, allowing for better downstream conditional analyses compared to meta-analyses combining multiple smaller studies. Furthermore, the availability of longitudinal electronic health record (EHR) data on untreated serum lipid levels allowed for more reliable lipid-phenotyping, resulting in decreased phenotypic variance and increased power to detect associations. This study identified a total of 171 genome-wide significant loci in the GERA data, 125 of which replicated known loci and 46 of which were novel. An additional 60 novel loci were identified through a GERA and GLGC meta-analysis. The authors suggested candidate genes for some of the novel loci as several have biological support for a role in lipid metabolism, including *SLCO1B1* and *BMAL1* for TG, *NR1H4* for TC and LDL-C,

UGT2B15/17 for TC, and *GNMT* for HDL. However, further studies are necessary to determine if these are indeed the causal genes at their loci.

Exome arrays, like GWAS arrays, allow for SNP genotyping, yet only in transcribed regions of the genome. This allows for more sensitive detection of associations with protein-coding and rare variants compared to GWAS arrays, and also makes candidate gene identification more straightforward given the coding nature of the variants. Recent exome-wide genetic studies, in addition to identifying a number of novel loci in recent times, have also provided additional layers of information regarding loci identified through GWAS. Liu et. al. [11] recently performed a large scale exome-wide association study using the HumanExome BeadChip array in over 300,000 subjects and identified 75 novel lipid loci. The authors functionally validated coding mutations found in *JAK2* (p.Val617Phe) and *A1CF* (p.Gly398Ser) that were found to associate with LDL-C and TG, respectively. Notably, the *JAK2* p.Val617Phe mutation was recently associated with CAD in cases of clonal hematopoiesis of indeterminant potential (CHIP), wherein a hematopoietic stem cell acquires this somatic mutation late in life, causing an overrepresentation of the mutant clone in the overall population of circulating hematopoietic cells [12]. With respect to *A1CF*, the authors performed rescue experiments with wild-type and mutant *A1CF* in hepatoma cells lines, and created a mouse with knock-in of the p.Gly398Ser mutation, to prove that this mutant allele increases plasma TG and APOB100 levels. This study also demonstrated that exome-wide association studies can be leveraged to provide additional information about GWAS loci. While previous GWAS identified a signal in the *PLEC1* gene, with the lead SNP located in an intron of *PLEC1*, the lead SNP from exome-wide studies was a coding variant in a nearby gene *PARP1*. Subsequent conditional analysis revealed that the significance of the intronic SNP was conditional upon the coding variant, but the coding variant was not conditional upon the intronic SNP, suggesting that the causal gene at that locus is *PARP1*. Meanwhile, an exome-based study by Lu et. al. [13] in 47,532 East Asians resulted in identification of three novel loci (located in the *ACVR1C*, *MCU*, and *CD163* genes); a combined meta-analysis with GLGC samples further resulted in identification of an additional nine novel loci, including coding variants in *FAM114A2*, *MGAT1*, *ASCC3*, and *PLCE1*. The fact that this study identified multiple novel loci even with significantly fewer subjects in the initial cohort highlights the necessity of expanding these genetic studies into non-European populations.

Most recently, Klarin et. al. [14] performed a large GWAS using data from >300,000 individuals from the Million Veterans Program by separately analyzing populations by ancestry (European, African, Hispanic), and then performing a meta-analysis using all three populations. As the largest GWAS to date (Figure 1) [6,8–11,13,14], this study identified 306 significantly-associated loci (nearly double that of the GLGC meta-analysis study), 188 of which were previously reported by GLGC studies and others, and an astounding 118 of which were novel loci that were replicable in the GLGC data. The group also conducted a focused analysis of predicted loss-of-function variants, and was able to identify a novel association of a stop-gain variant in *PDE3B* with increased plasma HDL-C and decreased TG. An individual homozygous for this mutation was found to have high HDL-C and low TG levels without taking lipid-lowering drugs, providing further support that *PDE3B* is an important regulator of these plasma lipids. The authors also point out some biological basis

for this association: treatment with celastrol, a phosphodiesterase inhibitor that does inhibit PDE3B, is known to cause increased blood levels of HDL-C and decreased TG [15]. While there is evidence in the literature suggesting that this can be caused through PDE3B regulation of lipolysis through insulin signaling [16], *PDE3B*'s role remains to be functionally validated. Indeed, the causal genes at most GWAS loci are unknown, making the data generated by this study replete with opportunities for future investigations of genes that potentially regulate plasma lipids.

Functional validation of plasma lipid GWAS loci:

Unbiased genetic screens have been widely successful in identifying novel genomic lipid loci. However, the rate of discovery is greatly outpacing the elucidation of their functional role in lipid regulation. Thus, it is increasingly important to dedicate studies aimed at confirming the functional relevance of the associated SNPs and/or nearby genes in the identified associations, a process we refer to as “functional validation.” Indeed, while some loci contain genes that have been previously implicated in lipid metabolism and are likely causal at the locus, the majority of identified loci have no clear functional relevance at discovery. This is in part due to the observation that most GWAS SNPs reside in noncoding genomic regions. As noncoding DNA is largely comprised of gene regulatory elements, these SNPs are widely thought to alter the transcription of nearby genes via enhancer element disruption. This is sometimes indicated by the presence of expression quantitative trait loci (eQTLs), where a certain SNP allele associates with increased or decreased expression of a nearby gene. Functional validation of loci usually consists of the identification of candidate causal genes, often either the closest gene or a gene linked to the SNP by an eQTL in a metabolic tissue such as the liver, adipose, or macrophage, and subsequent functional studies of those candidate genes in relevant cell and animal models.

To date, several loci have been functionally validated following their identification in GWAS, and these studies are outlined in Table 1 [8,11,17–38]. Among the earlier functionally validated loci, *PPPIR3B* was shown to have a role in plasma lipid regulation through hepatic overexpression in mice [8], while *GALNT2* [17,39], *TTC39B* [20], and *TRIB1* [18,19] have been further successfully validated in whole-body and/or liver-specific knock-out mice as well as studied in depth for mechanism (discussed in more detail in previous reviews [40,41]). *KLF14* is another remarkable gene that has been validated in genetic mouse models, and interestingly also has evidence for therapeutic potential [21]. As mentioned earlier, coding variants in *JAK2* and *AICF* have been validated in mouse models, and, very recently, a preprint reports that *AICF* deficiency causes decreased hepatic VLDL through an increase in ER stress pathways, and this effect appears to be APOBEC independent [24].

Some loci, such as those in the 1p13 and 19p12 chromosomal loci, are extremely complex, with multiple nearby genes and eQTLs, making deconvolution of the signal and identification of causal genes and mechanisms difficult. Extensive studies have implicated *SORT1* as the most likely causal gene for the signal in 1p13, and this fascinating locus is discussed elsewhere in this issue [42]. The GWAS signal in 19p12 falls within an intron of *SUGPI*, which codes for a putative splicing factor, and functional studies provide support

that *SUGP1* may be a causal gene at this locus as overexpression of hepatic *Sugp1* in mice results in increased TC, HDL-C, and TG [23]. However, previous studies showing that knockout of another nearby gene, *TM6SF2*, results in decreased TC and LDL-C [22] suggest that this may be the actual causal gene. These studies highlight the possibility that some GWAS signals are perhaps functionally comprised of more than one causal gene, and studies of these loci may have to contend with competing functions and directionalities of the effected genes.

Notably, while there have been multiple successful functional studies of plasma lipid loci, the field has seen a dearth of validations in recent years. This is not to say that candidate genes identified in lipid genetic studies are being neglected, but rather that validation studies have failed to demonstrate strong lipid phenotypes. For example, a GWAS signal located in an exon of *SLC39A8* is associated with HDL-C. However, global knockout of *Slc39A8* in mice did not result in significant changes in plasma HDL-C, although it did result in decreased tissue manganese levels, consistent with an association found in other GWAS [43]. This study highlights the risk that human genetic discoveries may not translate to mouse physiology, which, combined with uncertainty around the identity of candidate genes at many loci, makes GWAS functional validation studies quite challenging. Increased use of human cell lines, such as human induced pluripotent stem cells or even relevant immortalized cell lines, may help identify true causal genes that can then be further investigated in relevant animal models [44].

Long noncoding RNAs are potential causal elements at lipid GWAS loci:

The majority of GWAS SNPs associated with plasma lipid traits in humans lie in non-coding regions, yet functional validation of these loci is often focused on protein-coding genes or their corresponding transcriptional regulatory elements. However, these non-coding regions also harbor thousands of non-coding RNA molecules, and the disease-associated SNPs in these regions alter the expression of the non-coding RNAs [45], suggesting that these RNA molecules deserve to be the subject of functional interrogation at GWAS loci. This is particularly true for long non-coding RNAs (lncRNAs), which are by definition RNA molecules of >200bp length that do not code for functional peptides. LncRNAs can perform a variety of functions in both the cytoplasm and nucleus, and, intriguingly, the most genetically influential GWAS locus for coronary artery disease, the 9p21 locus, owes its functional relevance to the lncRNA *ANRIL* [46]. A recent report found via genome-editing studies in induced-pluripotent stem cells that the risk 9p21 haplotype actually alters the splicing of *ANRIL* [47]. Multiple recent papers have highlighted functions for lncRNAs in lipid metabolism, and these findings suggest that they warrant more focus in functional follow-up of human genetic screens [48].

The recently reported lncRNA *CHROME* (Cholesterol Homeostasis Regulator of miRNA Expression) lies in a genomic region that has previously been linked to both CAD and HDL-C [49]. *CHROME*, which is conserved among primates but not in other vertebrates, was found to be highly expressed in the plasma and atherosclerotic plaques of humans with CAD, with plaque expression localizing to inflammatory cells [50]. The researchers observed that cholesterol loading increased *CHROME* expression in hepatocytes and

monocytes, both in vitro and in vivo, and found that this increased expression was due to LXR activation. Knockdown of *CHROME* in HepG2 hepatoma cells reduced efflux of cholesterol to APOA1, a model of HDL biogenesis, due to decreased ABCA1 protein levels. Similar results were observed in knockdown of *CHROME* in THP-1 monocytic cells, suggesting that *CHROME* regulates macrophage cholesterol efflux to HDL. Finally, the investigators showed that *CHROME* binds to multiple microRNAs (miRNAs), such as miR-33a, miR-33b, miR-27b and miR-128, all of which have well defined roles in suppressing HDL biogenesis and cholesterol efflux. Induction of *CHROME* expression causes a downregulation of the miRNA expression, resulting in increased HDL biogenesis in hepatocytes and increased cholesterol efflux in macrophages. Thus, *CHROME* appears to be a potent regulator of HDL metabolism in multiple tissues, with its primary mechanisms mostly centered on non-coding RNA biology.

CHROME is not the only LXR-inducible lncRNA to be identified as a regulator of cholesterol metabolism. Transcriptomic analysis of primary mouse hepatocytes treated with an LXR agonist revealed robust increases in the expression of *LeXis* (Liver-expressed LXR induced sequence), a lncRNA located in close proximity to mouse *Abca1* (although under the control of an independent promoter) [51]. Hepatic overexpression of *LeXis* in mice reduced plasma cholesterol in a *Ldlr*-independent, Srebp-dependent manner, and *LeXis* knockout mice had the opposite phenotype of increased serum cholesterol and increased cholesterol biosynthetic gene expression. It was determined that *LeXis* interacts with the ribonucleoprotein RALY, itself an activator of cholesterol biosynthetic gene expression, and further studies showed that viral-overexpression of *LeXis* reduced atherosclerosis in *Ldlr* KO mice fed western-type diet [52]. Subsequent to the identification of *LeXis*, the same group identified *MeXis* (macrophage-expressed LXR induced sequence) via similar means in mouse peritoneal macrophages [53]. *MeXis* positively regulates the expression of *Abca1* through interaction with DDX17, a nuclear receptor coactivator that participates in LXR-induction of *Abca1*, and transplant of *MeXis* KO bone marrow in *Ldlr* KO mice accelerates atherogenesis compared to mice receiving wild-type marrow [53]. Finally, both *LeXis* and *MeXis* exhibit moderate conservation from mice to humans, and the human *MeXis* homolog contains a GWAS SNP with moderate, but sub-genome wide, significance to CAD in humans [53,54]. Thus, three separate lncRNAs mediate LXR-dependent effects on HDL lipoprotein metabolism in multiple tissues. Two of these lncRNAs have moderate genetic evidence supporting their relevance in human lipoprotein metabolism and CAD.

Given the striking examples outlined above, how likely is it that lncRNA function underlies GWAS signals for plasma lipid metabolism? There is considerable circumstantial evidence in support of this proposition. With respect to plasma lipid parameters, a 2016 study using 15 GWAS datasets found that 124 annotated human lncRNAs harbor at least one GWAS SNP associated with total cholesterol levels, while 90, 81, and 72 lncRNAs harbored GWAS SNPs for HDL-C, LDL-C, and TGs, respectively [55]. Interestingly, SNPs in the above mentioned *TRIB1* GWAS locus lie in the promoter of a lncRNA dubbed *TRIBAL* [56]. Douvris et al. were able to show that these GWAS SNPs affect *TRIBAL* promoter elements in in vitro luciferase assays, yet they were unable to find a functional role for *TRIBAL* in limited in vitro testing with knock-down and overexpression techniques. These observations all suggest that lncRNAs should be considered when investigating functional elements at

genetic loci for plasma lipid metabolism, and that these lncRNAs must be subjected to intense functional characterization [57], similar to that commonly applied to coding genes, before their causal role can be ruled out.

Conclusions:

Given the residual risk of cardiovascular disease in global populations despite the widespread success of statins and other lipid-lowering therapies, and the increasing risk due to metabolic syndrome, there remains a need for novel therapeutic interventions in the treatment of dyslipidemia and CAD. Unbiased human genetic screens such as GWAS and whole-exome sequencing have proven to be incredibly powerful in identifying novel regions in the genome that participate in disease pathogenesis. Given the recent studies outlined in this article, there are now hundreds of loci implicated in the regulation of lipid metabolism in humans. However, the discovery of these loci has greatly outpaced the translation of this information into novel molecular mechanisms. While some genes, such as *GALNT2*, *TRIB1*, and *SORT1*, have confirmed roles in lipid metabolism, the majority of GWAS loci have no function assigned. Functional validation is limited by the difficulty and costs of the experiments, the risk of poor translation between mouse physiology and human genetics, and the uncertainty of the relevant functional element to test. To this latter point, we must increasingly consider lncRNAs as a potential source of functional translation of GWAS loci. Overall, robust functional validation of potential GWAS hits must continue, using a variety of relevant models and techniques [41,44], for the field to begin to fully appreciate the deluge of novel regulators of lipoprotein metabolism unearthed by unbiased human genetic screens.

Acknowledgements:

Funding for this work was received from the National Institutes of Health (NIH)

Financial support and sponsorship:

This work was supported by funding from the Division of Cardiology, Department of Medicine at Columbia University, and grants from the American Heart Association (16SDG31180039 to R.C.B.) and NIH (R01HL141745 to R.C.B.).

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KEY POINTS:

- Given the residual risk of cardiovascular disease and increasing risk of metabolic syndrome throughout global populations, the identification of novel regulators of lipid metabolism could help spur novel therapeutic development.
- Unbiased genetic screens, such as genome-wide association studies, have proven to be incredibly powerful for identifying novel regions of the genome involved in dyslipidemia and coronary artery disease pathogenesis. To date, hundreds of loci have been identified that presumably harbor functional mediators of lipid metabolism.
- Loci discovery has greatly outpaced their subsequent functional validation. Only a handful of loci now have known mechanisms in lipid metabolism. Successful elucidation of lipid-relevant mechanisms of genes such as *GALNT2*, *SORT1*, *TRIB1*, and *TTC39B*, have proven to be the exceptions, not the rule.
- Most functional analysis of genetic loci focuses on protein coding genes. However, recent reports demonstrating the functional relevance of long noncoding RNAs in lipid metabolism combined with the observation that many GWAS loci harbor noncoding RNA transcripts, suggests that these elements must be included in translational studies of these loci.

Figure 1. Select plasma lipid genetic studies from 2008 to present and notable associations. The use of unbiased human genetic screens to identify plasma lipid metabolism loci in the genome has identified >300 loci in the last decade. * indicates genes for which some in vivo animal validation exists. Total: total identified loci from study. Novel: loci identified in the study that were not previously identified in plasma lipid genetic studies, as reported by the study authors.

Table 1.
Published In Vivo Functional Validation Studies of Plasma Lipid GWAS Loci.

This table summarizes published in vivo functional studies of genes identified through GWAS as being regulators of lipid metabolism. Lead SNPs and trait associations are drawn from the 2018 Klarin et. al. GWAS study [13], with traits listed in order of smallest to largest p-value. CAD associations are taken from the 2013 GLGC GWAS study [9] supplemental info unless otherwise indicated.

Lead SNP	Chr	GWAS Lipid Association	Validated Gene(s)	Validation models	Validation Result	Proposed Mechanisms	CAD Association	Other Associations
rs4846914	1q42	HDL-C, TG	GALNT2	Whole Body and Liver-specific mouse KO ¹⁷	Decreased HDL-C (LSKO), Decreased HDL-C and increased TG (WB)	Glycosylation of Plp, Angptl3, and ApoCIII	Yes	
rs58253018	8q24	TG, TC, LDL-C, HDL-C	TRIB1	Liver-specific mouse KO ¹⁸ Liver-specific mouse OE (AAV) ¹⁹	Increased TC, non-HDL-C, TG Decreased TC, HDL-C, LDL-C, TG	May be regulating VLDL secretion and may involve interaction with the transcription factor C/EBPα.	Yes	Adiponectin ²⁸ , Liver enzymes ²⁹
rs686030	9p22	HDL-C	TTC39B	Whole Body mouse KO ²⁰ Whole Body mouse KO on Ldlr ^{-/-} background (Western Diet) ²⁰	Increased HDL-C Decreased TC (males) and LDL-C (males and females), Increased HDL-C in males.	Induction of Lxr protein in setting of Ttc39b deficiency and increased expression of intestinal <i>Akca1</i> .		Endometriosis ³⁰
rs4841132	8p23	HDL-C, TC, LDL-C, TG	PPP1R3B	Liver-specific mouse OE (AAV) ⁸	Decreased HDL-C			TIBC ³¹ , NAFLD ³²
rs6971365	7q32	HDL-C, TG	KLF14	Liver-specific mouse KO ²¹	Decreased HDL-C	Regulation of ApoA-I production in hepatocytes	Yes	T2D ³³
rs140868651	19p13	TG, LDL-C, TC	TM6SF2	Liver-specific mouse OE ²² Whole Body mouse KO ²²	Increased LDL-C, TC Decreased TC and HDL-C, Increased TG	Regulation of cholesterol biosynthesis	Yes	NAFLD ³²
rs41274050	10q11	TC, LDL-C, TG	SUGP1	Liver-specific mouse OE (non-viral construct) ²³	Increased TC, TG, and HDL-C	Regulation of Hmgcr activity through splicing		
rs77375493	9p24	HDL-C, TC, LDL-C	AI1CF	Whole Body mouse KO and LOF mutant ²⁴	Increased TG	Regulation of VLDL secretion		eGFR ³⁴
rs611917	1p13	LDL-C, TC, TG	JAK2 [*]	Chimeric Bone-marrow Jak2 p-Val617Phe mice on Ldlr ^{-/-} background (western diet) ¹¹ Liver-specific mice OE (AAV) ²⁵	Decreased LDL-C Decreased TC		Yes ¹²	Myeloproliferative disease ³⁵ , Thrombosis ³⁶
rs1260326	2p23	TG, TC, LDL-C	GCKR	Whole Body mouse KO on Ldlr ^{-/-} background ²⁵ AAV8 OE in <i>ob/ob</i> and HFD-fed mice ²⁶ Liver-specific rat OE ²⁷	Decreased VLDL secretion Increased TG	Regulation of LDL uptake and LDL catabolism. Regulation of ApoB trafficking	Yes	Programulin ³⁷ NAFLD ³² , T2D ³⁸

Abbreviations: CAD, coronary artery disease; KO, knock out; OE, over expression; HFD, high fat diet; AAV, adeno-associated virus; LOF, loss of function; TIBC, total iron blood content; NAFLD, non-alcoholic fatty liver disease; T2D, type 2 diabetes; eGFR, estimated glomerular filtration rate.

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* Functional studies of Jak2 are notably focused on the p.Val617Phe variant.

[‡] Multiple additional animal models for Sort1 exist, as reviewed in 2014 Strong et. al. [41].