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Proceeding Paper

In silico study of the functional effects of polymorphic loci associated with the risk of developing uterine leiomyomas according to genome-wide studies

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Abstract: Uterine leiomyoma (UL) is the most common benign tumor causing considerable morbidity during the reproductive years in women with contributions from environmental and genetic factors. According to the GWAS studies, there are many genes and polymorphisms that related with, somehow responsible for the UL pathogenesis, but the biological mechanisms underlying this association remain unclear. This study aimed to investigate the published GWAS studies of UL to recognize significant functionality of *TNRC6B* polymorphism linked with UL. Six SNPs were selected based on the 7 GWAS published of their association with UL by PubMed database. For their analysis, including their epigenetic effects, expression and splicing patterns, using in silico approach and bioinformatics tools (HaploReg, GTEx-portal and Gene Ontology Resource). Based on HaploReg, several epigenetic effects regulating these SNPs were found as: rs12484776(1 motif changed, 16 enhancers and 4 DNAs histone markers), rs4821939(3 motifs changed, 11 enhancers, 4 protein bounds and 5 DNAs histone markers), rs733381(2 motifs changed, 11 enhancers, 1 protein bound and 2 DNAs histone markers), rs12484951(1 motif changed histone markers), rs3830738 (5 motifs changed histone markers) and rs17332320(2 motifs changed and 2 DNAs histone markers). Depending on GTEx, inferred that (rs12484776, rs4821939, rs733381, rs3830738, rs12484951 and rs17332320) are associated with the expression of genes/in tissues as: (4/4, 4/4, 4/4, 3/3, 4/4 and 2/2), respectively. These loci do not regulate the expression level of any genes in the UL pathophysiology important tissues, and are not associated with the alternative splicing traits (sQTL) of any gene in any tissue. By Gene Ontology Resource, indicated that no statistically significant biological pathways for genes associated with the studied polymorphisms have been identified. The in-silico analysis of GWAS *TNRC6B* gene polymorphisms significant for fibroids have pronounced epigenetic effects and affect the expression of six genes (*RP51042K10.10*, *FAM83F*, *TNRC6B*, *RP51042K10.13*, *SLC25A17* and *XPNPEP3*), which may be the basis of their involvement in the pathophysiology of fibroids.

Keywords: bioinformatics; in silico analysis; uterine leiomyoma; *TNRC6B* gene.

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1. Introduction

The most frequent tumors of the female reproductive system are uterine leiomyomas (ULs)[1,2]. They originate from the myometrium and are benign monoclonal uterine smooth muscle tumors[3,4]. By the time of menopause, more than 70% of women have uterine fibroids. With a lifetime prevalence of 30% to 70%[5], they afflict 20% to 40% of women of reproductive age[4] (they frequently regress after menopause). Around a quarter of women with UL are aware of their symptoms, despite the fact that many of them are oblivious of them[6]. and possible reproductive disruption, in addition to other indications of the overall effect of fibroids on health-related quality of life. Miscarriage is up to twice as prevalent

in women with symptomatic UL, and complications affect 10% to 40% of pregnancies with UL[7].

Uterine leiomyoma is a complicated condition caused by a number of demographic, dietary, and hormonal risk factors[8–10] as well as biological, epigenetic, and genetic causes[11], with the genetic component accounting for 40–50%[12] of the illness. The genetic foundation for the formation, development, and progression of ULs is now being studied by a considerable number of academics and researchers.

Utilizing the whole genome association search (GWAS), researchers may investigate how genetic factors contribute to the emergence and development of various multifactorial diseases, including ULs[13]. On the basis of GWAS, several research teams are actively researching the genetic basis of ULs. In addition, there is a lack of consistency in the findings among various global groups and they are little repeated and sometimes contradictory. It was suggested by many candidate gene association studies that *TNRC6B* was linked to UL in several ethnic populations. However, the biological mechanisms behind these relationships are still largely unclear. In addition to making it possible to choose genetic markers for a study with greater accuracy and support, the exponential growth of biomolecular data and its mining into databases have also made it possible through providing tools for comprehensive analysis to gain deeper insights into the potential functions of candidate genetic variants and the mechanisms by which they contribute to traits[14-16]. A thorough in silico analysis of the *TNRC6B* polymorphisms, which were found to be linked with UL, was carried out using numerous online genomic databases and recent advancements in bioinformatics. The goal of this bioinformatic investigation was to gain knowledge of the mechanisms behind these linkages. This study aimed to investigate the published GWAS studies of UL to recognize significant functionality of *TNRC6B* polymorphism linked with UL.

2. Materials and Methods

2.1. Selection of polymorphisms:

Based on the results of their connection with UL that have been published, polymorphisms were chosen for the study. The phrases "uterine leiomyoma," "*TNRC6B*," and "in silico analysis" were used in different combinations to search PubMed for relevant articles. The search showed up 7 articles that were pertinent. These papers listed a total of six SNPs connected to UL in 4 ethnic samples: Japanese, European, UK and European & African. Table 1 contains a list of the chosen polymorphisms.

2.2. Bioinformatic analysis:

The following bioinformatics tools were used for the analyses: Utilizing the integrated online program HaploReg v4.1[17], chosen polymorphism loci were examined for their functional importance (chromatin states, motifs changes, protein interactions, regulatory potential, and eQTLs).

In addition to HaploReg (v4.1), the GTExportal data (http://www.gtexportal.org) was used to evaluate the influence of the six candidate SNPs for UL on gene expression level (cis- and trans-eQTL) in organs and tissues[18]. Using the Gene Ontology Resource tools available at (http://geneontology.org)[19], the functional importance of the candidate genes for UL in the different biological pathways was investigated.

3. Results

3.1. Genomic location of the SNPs:

In the introns of the *TNRC6B* gene, six reference SNPs were found.

3.2. Regulatory effects:

They suggest that all reference SNPs can produce various regulatory effects, albeit to a different extent, as follows:

SNP rs12484776, displays histone marks associated with enhancers in 16 tissues(hESC Derived CD56+ Mesoderm Cultured Cells, and Primary B and T cells (regulatory, effector/memory enriched, helper, etc.) from peripheral blood and brain (hippocampus middle, anterior caudate, dorsolateral and prefrontal cortex, etc., and male fetal brain, fetal adrenal gland, fetal muscle trunk, etc.)), located in the DNase-1 hypersensitive region in 4 tissues (H1 Derived Mesenchymal Stem Cells, Foreskin Fibroblast Primary Cells skin01, Fetal Lung and Placenta), and altered motif (SRF) for the transcription factor.

SNP rs4821939, displays histone marks associated with enhancers in 11 tissues(hESC Derived CD56+ Mesoderm Cultured Cells, and Primary B and T cells (regulatory, effector/memory enriched, helper, etc.) from peripheral blood and brain (hippocampus middle, anterior caudate, dorsolateral and prefrontal cortex, etc., and male fetal brain, fetal adrenal gland, fetal muscle trunk, etc.)), located in the DNase-1 hypersensitive region in 4 tissues (Primary T cells from cord blood, Primary hematopoietic stem cells G-CSF-mobilized Male and female, and HUVEC Umbilical Vein Endothelial Primary Cells), 5 bound proteins(iPS-15b Cells, Primary hematopoietic stem cells G-CSF-mobilized Male and female, and Monocytes-CD14+ RO01746 Primary Cells) and 3 altered motifs (Hmx,Mef2 and Nkx2) for the transcription factor.

SNP rs733381, displays histone marks associated with enhancers in 11 tissues(hESC Derived CD56+ Mesoderm Cultured Cells, and Primary B and T cells (regulatory, effector/memory enriched, helper, etc.) from peripheral blood and brain (hippocampus middle, anterior caudate, dorsolateral and prefrontal cortex, etc., and male fetal brain, fetal adrenal gland, fetal muscle trunk, etc.)), located in the DNase-1 hypersensitive region in 2 tissues (Fetal Kidney and ovary), bound proteins(ZNF263) and 2 altered motifs (BCL and p300) for the transcription factor.

SNP rs12484951, displays histone marks associated with 2 altered motifs (BATF and Hmbox1) for the transcription factor. SNP rs3830738, displays histone marks associated with 5 altered motifs (Foxj1, Foxk1, Irf, TCF12 and p300) for the transcription factor.

SNP rs17332320, displays histone marks located in the DNase-1 hypersensitive region in 2 tissues (BLD and BLD) and 2 altered motifs (HDAC2 and Pax-5) for the transcription factor.

3.3. Expression QTLs:

In 4 tissues (organs), 4 SNPs seemed to have a cis-eQTL influence on the expression of 4 genes (*RP51042K10.10, TNRC6B, RP51042K10.13*, and *XPNPEP3*). However, SNP rs3830738 only affected the expression of 3 genes (*RP51042K10.10, TNRC6B*, and *RP51042K10.13*), while SNP rs17332320 only affected the (*RP51042K10.10* and *TNRC6B*).

3.4. The alternative splicing traits (sQTL):

The GTEx dataset highlighted the regulatory function of mRNA precursor splicing patterns. According to GTEx, none of the six SNPs are linked to alternative splicing traits (sQTL) of any gene in any tissue.

3.5. Pathway analysis:

This investigation was performed on *TNRC6B* since it was discovered to be linked with UL and multiple reference polymorphisms were mapped to this gene, as well as because several reference SNPs may impact the expression of this gene according to the eQTL analysis. The following genes were evaluated using the Gene Ontology database: (*RP51042K10.10, FAM83F, TNRC6B, RP51042K10.13, SLC25A17*, and *XPNPEP3*) in table 1, where no statistically significant biological pathways for genes related with the researched polymorphisms were discovered.

Table 2. Overview about functional effects of *TNRC6B* gene polymorphisms at (22q13.1) associated with UL in GWAS studies.

Gene	SNPs (Pos.)	OR (effect allele), *p* [Ref.]	Regulatory effects	eQTL
TNRC6B	rs12484776(40256869)	OR=1.23 (G), p=2.8 × 10^{-12} [20]	Enhancer 16 tissues, DNAse 4 tissues, 1 altered motif	*RP51042K10.10, TNRC6B, FAM83F RP51042K10.13, XPNPEP3, SLC25A17*
		OR=0.89 (A), p=4.6× 10^{-18} [21]		
	rs4821939(40263247)	OR=1.08 (A), p=7.8 × 10^{-16} [22]	Enhancer 11 tissues, DNAse 4 tissues, 5 Protein Bounds, 3 altered motifs	*RP51042K10.10, FAM83F, TNRC6B, RP51042K10.13, SLC25A17, XPNPEP3*
	rs733381(40273644)	OR=1.10 (G), p=5.7 × 10^{-11} [23]	Enhancer 11 tissues, DNAse 2 tissues, 1 Protein Bound, 2 altered motifs	*RP51042K10.10, FAM83F, XPNPEP3 RP51042K10.13, TNRC6B, SLC25A17*
	rs12484951(40307071)	OR=1.11 (G), p=3.2 × 10^{-13} [24]	2 altered motifs	*RP5-1042K10.10, FAM83F, SLC25A17, RP5-1042K10.13, TNRC6B, XPNPEP3*
	rs3830738(40315223)	OR=0.91 (A), p=2.7 × 10^{-13} [25]	5 altered motifs	*RP5-1042K10.10, TNRC6B, FAM83F, RP5-1042K10.13, XPNPEP3*
	rs17332320(40315616)	OR=1.15 (T), p=1.6 × 10^{-12} [26]	DNAse 2 tissues, 2 altered motifs	*RP51042K10.10, TNRC6B, SLC25A17, RP5-1042K10.13, XPNPEP3, FAM83F*

4. Discussion

This study shows that, in addition to the previously reported TNRC6B gene as being connected with UL, many reference polymorphisms were mapped to this gene, and that the expression of this gene may be influenced by several reference SNPs based on the eQTL analysis. These associated polymorphism loci were shown to have no major functional role (multiple expression and splicing patterns) that was reported to correlate with UL, that somehow did not affect any gene in any tissue, by documenting pronounced pleiotropic tissue-specific regulatory/expression/splicing effects.

In general, the degree of gene pleiotropy appears to be inversely linked to the gene's proportionate contribution to the trait. Given that the majority of genes in the human genome are pleiotropic[27], the predicted contribution of each to a specific characteristic is relatively small. As a result, extremely pleiotropic genes have a limited impact size and frequently provide false negative findings in GWAS unless their contribution to a specific trait is greater than the average for other traits. The current analysis also offers light on often observed discrepancies in related polymorphisms and failed attempts to replicate potential loci in other ethnic populations.

5. Conclusions

The in-silico analysis of GWAS *TNRC6B* gene polymorphisms significant for fibroids have pronounced epigenetic effects and affect the expression of six genes (*RP51042K10.10*, *FAM83F*, *TNRC6B*, *RP51042K10.13*, *SLC25A17* and *XPNPEP3*), which may be the basis of their involvement in the pathophysiology of fibroids.

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Institutional Review Board Statement: This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Local Ethical Committee of Belgorod State University.

Data Availability Statement: The data generated in the present study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

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