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Whole-Genome Sequencing Identifies Novel Heterozygous Mutation in *ALMS1* in Three Men with Both Peyronie's and Dupuytren's Disease

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Abstract

Peyronie's Disease (PD) is estimated to occur in up to 13% of males and has been associated with Dupuytren's Disease (DD). We identified three men with PD/DD and hypothesized that there may be a genetic association between the two diseases. Blood samples were collected from the participants and sent for whole genome sequencing. A rare non-synonymous mutation in the *ALMS1* gene was identified in three men. Interestingly, *ALMS1* is associated with TGF-b and aberrant fibrosis. This pilot study generates the hypothesis that mutations in *ALMS1* may predispose patients to development of PD/DD.

Keywords

Peyronie's Disease (PD); Dupuytren's Disease (DD); Genetics; WGS

INTRODUCTION

Peyronie's disease (PD) is an acquired superficial fibrotic disease of the tunica albuginea. The hallmark symptom of PD is abnormal penile curvature, resulting in pain, erectile dysfunction, and emotional distress[1]. The estimated prevalence of PD ranges from 0.3–13.1% of males worldwide[2]. Despite being a relatively common disorder, the pathophysiology of PD is not fully understood[3]. Microtrauma to the erect penis is hypothesized as the most common cause of PD[3]. PD is linked to other fibrotic diseases. Notably, PD and Dupuytren's Disease (DD) have been linked since 1828[4]. Both are diseases of aberrant fibrotic tissue impacting the physiological function of the body[4]. Research has shown a link between the two diseases and familial (father-to-son) transmission of both conditions[5].

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While the two diseases have been linked in families, the genetic basis for the abnormal fibrotic deposition is still not full elucidated. Further genetic investigations are necessary to identify possible genetic associations of PD and DD[6]. This study evaluated the genetic mutations linked to PD and DD in three non-familial men with both diseases.

MATERIALS AND METHODS

Patient Selection

Patients were identified through retrospective chart review using ICD codes for PD and DD. Nine males met these criteria, and three agreed to participate in the study. The three participants shared no known familial relation and had no recorded familial history of PD/DD. All patients were identified at University of Miami Health System and provided informed consent to participate in the study. The University of Miami IRB approved this study (#20150740).

Whole Genome Sequencing

Research grade whole genome sequencing (WGS) was performed at the John P. Hussman Institute for Human Genomics Center for Genome Technology at the University of Miami. The QIAamp blood maxi kit (Qiagen, Germantown MD, USA) was used for whole blood DNA extraction. Following DNA quantification using the Qubit dsDNA Broad Range Assay (Thermo Fisher Scientific, Waltham, MA USA), and quality control on the TapeStation (Agilent, Santa Clara, CA USA), 1μg of DNA was used as input for the TruSeq PCR-free DNA HT sample preparation kit (Illumina, San Diego, CA USA). Resulting library quality was conducted using the Bioanalyzer 2100 (Agilent, Santa Clara, CA USA) to confirm the absence of free adapters, adapter dimers. and appropriate library size. Quantification of libraries by real-time PCR was performed, followed by equimolar pooling, concentration normalization, and sequencing on an S4 flow cell of the NovaSeq 6000 (Illumina, San Diego, CA USA). Sequencing was performed using paired end 100bp reads targeting at least 30X genome coverage. Resulting raw FASTQ files were processed through a bioinformatics pipeline including alignment to the human reference genome GRCh38 and germline variant calling with the GATK-HaplotypeCaller[7].The processing workflow is modularized and consists of four stages: (1) Read mapping to GRCh38 with bwa-mem (v0.7.12); (2) BAM file production, duplicate read identification, and BAM file processing using PICARD-tools (v.2.1.1) and samtools (v1.3); (3) Re-calibration of base quality scores; (4) Genotyping variant calls using with the HaplotypeCaller to produce sample-specific genome-level (gVCFs) followed by joint genotype calling. Resulting variants were annotated with ANNOVAR software (v 2019-10-24) against the RefSeq gene model for hg38[8].

RESULTS

The genomes of the three individuals were sequenced to an average coverage of 33.8X, 36.6X, and 40.0X. Across all individuals, over 7.9 million single nucleotide and short insertion-deletions were identified. To prioritize potential functional candidate variants, we filtered to the 133,915 coding variants, further filtered only to only missense, nonsense, and frameshift variants with a population frequency in the gnomAD database of less than

0.05 (3469 variants). From this, we identified 16 candidate genes where all three individuals harbored a rare protein altering variant. These include *AK2, OTOF, ALMS1, NT5DC4, SH3TC1, PCDHA10, DMBT1, POTEB3, FANCA, TSPAN10, ROCK1, KMT2B, FCGBP, LAMA5, URB1*, and *USP18* (supplementary table 1). Each candidate gene was evaluated, through literature review, for known functions. Based on the literature, *ALSM1* was selected because of likely involvement in the PD/DD phenotype[9].

The three *ALMS1* variants included: rs41291187 (p.H624R), rs34071195 (p.K3435E), and rs45501594 (p.T3543S). Each had a coverage of at least 25X in all three individuals and an alternate allele frequency of at least 0.3 in the variants and 0 in the references, suggesting high confidence variant calls. All three *ALMS1* variants had less than 1% population frequency (Table 1)[10]. One potentially pathogenic point mutation in the *ALMS1* gene (centrosome and basal body associated protein) was confirmed by Sanger sequencing in the 1 patient carrier only. The other two samples were found to have poor sequencing reads and the mutation was not validated.

DISCUSSION

We identified three, non-familial males afflicted by PD/DD with rare missense mutations in *ALMS1.* A potential pathogenic point mutation variant was identified in the *ALMS1* gene in one participant. A comprehensive literature search on the genetics of PD/DD did not show any previous association between *ALMS1* and the PD/DD phenotype[6,11]

We hypothesized that *ALMS1* mutation leads to upregulation of TGF-b signaling, resulting in fibrosis and the PD/DD phenotype (Figure 1). *ALMS1* is associated with cell cycle control, ciliogenesis, and intracellular transport, and other cellular functions[12]. *ALMS1* mutations have been associated with growth factor beta/bone morphogenic (TGF-B/BMP) protein signaling[13]. Aberrant TGF-B/BMP signaling has been linked to increased fibrosis and PD/DD[14,15]. Additionally, mutations in *ALMS1* are responsible for Alström Syndrome, a constellation of symptoms that shares similarities with PD/DD. Alström Syndrome symptoms include metabolic defects, hearing/vision loss, and multiorgan fibrosis[9]. Our genetic analysis and the known association between *ALMS1* and TGF-b suggest a possible genetic link between ALMS1 and PD/DD that should be further explored.

It is important to note that the mutation was validated in only one sample. Sequencing reads in the other two samples were poor quality and unable to be used for mutational validation. The poor-quality sequencing was most likely secondary to sample DNA degradation. Although the specific mutation was not identified in all three samples, a protein coding mutation in the *ALMS1* gene found in all three participants and a specific point mutation identified in one participant. This study demonstrates that *ALMS1* is likely associated with PD/DD and further studies are necessary to elucidate specific mutational links between *ALMS1* and PD/DD.

This study represents a rare evaluation of patients with PD/DD by WGS. It should be noted that WGS does have limitations and is influenced by sample quality. Our study has a limited sample size of three patients which may impact the genetic mutations identified. The

potential pathogenic point mutation variant was only identified in one of the patient samples, limiting the generalizability of these results. It is important to complete larger genetic studies in the future.

CONCLUSION

Using WGS, we have identified a variant in the *ALMS1* as a possible genetic association between PD and DD in three non-familial males. This finding supports the value of using genetic tools, such as WGS, to investigate a genetic link between PD and DD. PD is an important cause of sexual dysfunction and psychological strain in the male population. It commonly co-occurs in patients with DD. Identification of a possibility pathogenic variation is an essential part of being able to offer patients disease counseling and refine future treatment options. These tools will help improve disease understanding and improve patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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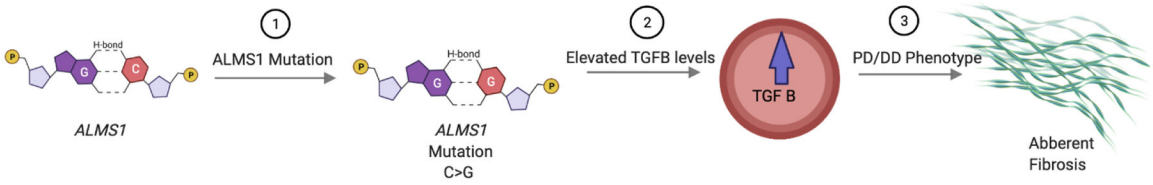

Figure 1.

ALMS1 Pathway. The proposed role of *ALMS1* in PD/DD via upregulation of TGF-B signaling leading to increased fibrosis.

Table 1.

ALMS1 mutation prevalence. The reported allele frequency for the three identified ALSM1 mutations.

***ALMS1* Variant (rsID)**	**Allele Frequency (gnomAD)**
rs45501594	0.006688
rs34071195	0.01628
rs41291187	0.01586