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14. ABSTRACT

Duchenne muscular dystrophy is among the most common genetic disorders and is caused by loss-of-function mutations in the *DMD* gene, leading to loss of the dystrophin protein in skeletal and cardiac musculature, as well as other cell types. In healthy individuals, dystrophin is expressed in skeletal muscle and heart at high levels in fetal life and does not change with age. In DMD patients, dystrophin is lost from fetal life onwards. We have shown that DMD skeletal muscle shows little cellular pathology in fetal life, but soon after birth there is broad activation of NFkB inflammation, followed by failure of myofiber metabolic differentiation, then progressive fibrofatty replacement of muscle and an early death. The presentation and progression of DMD is variable, in part due to genetic modifiers we and others have identified. Response to anti-inflammatory drugs is also variable (corticosteroids, vamorolone). The goal of the proposed research is to develop computational models drawing on multivariate data that are able to explain disease variation (onset, progression, and drug response) in DMD. The hypothesis to be tested is that integration of robust longitudinal clinical, biomarker, and pharmacological data in clinical trial settings will lead to validated multiscale models of DMD disease onset, progression and response to therapy. The patient resources to carry out this study are in hand and are from 168 DMD boys recruited into vamorolone clinical trials (all 4 to <7 years, steroid naïve at study entry). Longitudinal clinical outcomes, biomarker assessments (mutation, genetic modifiers, serum proteins), and pharmacokinetics will be used to develop the proposed models. Aim 1 is to complete the full data set through generation of serum biomarker data (somaSCAN 5,000 biomarkers. Aim 2 is to model drug response and validate models for both prednisone and vamorolone using an independent clinical trial (VBP15-004). Aim 3 is to model disease onset and progression, similarly validating the models for both prednisone and vamorolone using independent data (VBP15-004). Completion of the proposed aims is likely to have impact on DMD clinical trial conduct and interpretation, understanding of disease onset, progression, and drug response. In addition, it is anticipated that the integrated models will lead to age-resolved patient treatment models and new drug targets.

15. SUBJECT TERMS

Duchenne muscular dystrophy, pharmacogenomics, vamorolone, glucocorticoids, clinical trials, genetic modifiers

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1. Introduction

The goal of this CDMRP research proposal is to leverage recently completed clinical trials of vamorolone to better understand genetic modifiers of DMD disease severity, and also generate novel data regarding PGx modifiers of DMD drug response. The clinical trials provide robust data on motor outcome assessments, in a more homogeneous patient population than afforded by previous natural history and registry studies. This should increase the statistical power of genetic modifier studies, both in terms of defining association with baseline clinical severity, and treatment response. Furthermore, the vamorolone clinical trials provide pharmacokinetics data on over 150 DMD children, and this should be adequate for the first PGx studies of drug exposure and treatment response in DMD.

We will test the hypothesis that genetic modifiers of treatment response can be identified using these cohorts of homogenous and well-characterized DMD boys (VBP15-004; VBP15-002 clinical trials), and these will point to approaches for optimization of existing treatments (corticosteroids, vamorolone), as well as new pathways for alternative or complementary treatments.

2. Keywords

Duchenne muscular dystrophy, vamorolone, corticosteroids, pharmacogenomics, genetic modifiers.

3. Accomplishments

Below we list the proposed Specific Aims, and provide a progress report on each of the aims and subaims.

Specific Aim 1. Define genetic modifiers of baseline motor function, treatment response on motor outcomes, and drug exposure (pharmacogenomics) in DMD participants in the vamorolone clinical trials.

Aim 1A. Determine if established genetic modifiers are associated with baseline motor function in DMD boys in the vamorolone trials.

This subaim is largely complete.

The proposed data generation under this grant, Illumina Genome Diversity Arrays + PGx, genotyping of 1,933,117 genetic markers (loci) in each participant in the clinical trials was completed. As proposed, the DNA samples were prepared and aliquoted by AGADA BioSciences, then the samples sent to Mt Sinai genomic core laboratory for genotyping (SNP chips). The data was sent to collaborators Drs. Elena Pegoraro and Luca Bello in Italy for data processing and application of a data mask corresponding to the specific gene loci (SNPs) described in the grant. The curated genotype data was then sent to statistician Utkarsh Dang (Carleton University) for associations with baseline motor function (genetic modifiers).

This analysis showed that two genetic modifier loci, TCTEX1D1 and LTBP4, were robustly associated with baseline motor function in participants in the vamorolone trials (**Figure 1**). More limited associations were seen with ADRB2 and VDR polymorphisms.

❑Statistically Significant differences identified:

TCTEX1D1:

✓ rs1060575 (AA<TA): baseline TTSTANDV, TTCLIMBV and TTRWV

LTBP4:

✓rs2303729 (AA>GG): baseline TTRWV and NSAA

✓rs1131620 (AA<GG): baseline TTRWV and NSAA

✓rs1051303 (AA<GG): baseline TTRWV and NSAA

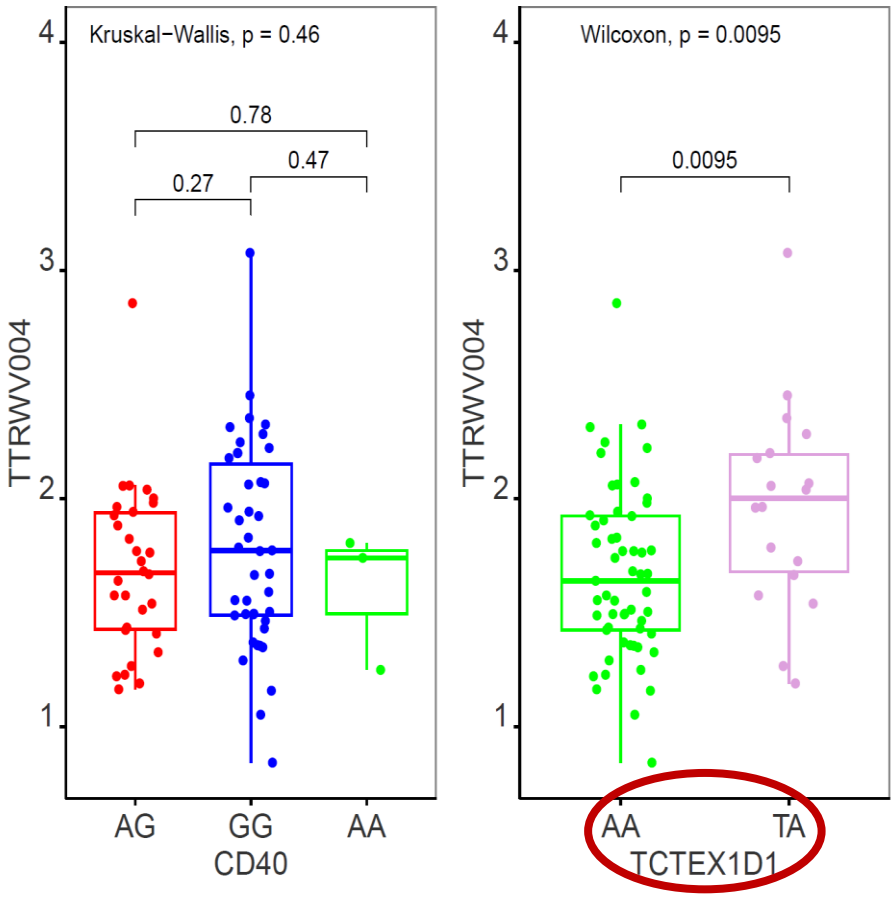
ADRB2 G16R:

✓rs1042713 (AA>GG): baseline TTCLIMBV

VDR Fok1:

✓rs2228570 (AG>GG): baseline TTSTANDV

Figure 1. Exemplar analysis of motor outcomes with genetic modifiers. Shown is Time to Run/Walk 10 meters velocity plotted in vamorolone clinical trial participants as a function of genotype at two loci (CD40, TCTEX1D1). CD40 shows no association of motor function with genotype, whereas TCTEX1D1 shows a strong association.



This is considered a strong positive result of this subaim.

Aim 1B. Determine if established genetic modifiers are associated with treatment response (efficacy and safety).

All data needed for this analysis is in hand, and this subaim will be completed in Year 1. The statistical analyses will be done by Utkarsh Dang and the PI (Eric Hoffman).

Aim 1C: Establish pharmacogenomics loci that are associated with vamorolone drug exposure (PK).

This subaim is largely complete.

Human genetic variation (polymorphisms) in genes coding proteins involved in the absorption, distribution, metabolism, and elimination (ADME) processes of drugs have a strong effect on drug exposure and the downstream efficacy and safety outcomes. Vamorolone, a first-in-class dissociative steroidal anti-inflammatory drug currently being investigated clinically for treating Duchenne Muscular Dystrophy (DMD), primarily undergoes oxidation by CYP3A4 and CYP3A5 and glucuronidation by UGT1A1. This work assesses the PK of vamorolone and sources of inter-individual variability (IIV) in 81 steroid naïve DMD boys aged 4 to <7 years old considering the genetic polymorphisms of CYP3A4*22, CYP3A4*1B, CYP3A5*3 and UGT1A1*60 utilizing population pharmacokinetic (PopPK) modeling. A one-compartment model with zero-order absorption (T_{k0}, duration of absorption), linear clearance (*CL/F*) and volume (*V/F*) describes the plasma PK data for DMD boys receiving a wide range of vamorolone doses (0.25~6 mg/kg/day). The typical *CL/F* and *V/F* values of vamorolone in DMD boys were 35.8 L/hr and 119 L, with modest IIV. The population T_{k0} was 3.14 hr yielding an average zero-order absorption rate (*ka*) of 1.16 mg/kg/hr with similar absorption kinetics across subjects at the same vamorolone dose (i.e., no IIV on T_{k0}). The covariate analysis showed that none of the genetic covariates had any significant impact on the PK of vamorolone in DMD boys. Thus, the PK of vamorolone is very consistent in these young boys with DMD.

There were PGx loci that showed trends with drug exposure in the anticipated directions. For example, UGT1A1 (the major metabolism pathway for vamorolone), has a loss of function allele at rs4124874, and DMD participants in the trials with the loss of function showed more rapid clearance, but this did not reach statistical significance (p=0.1). The lack of statistical significance is likely due to lack of statistical power due relatively limited data and high variability in the PK measures. Specifically, we had some drop out of participants in this analysis, as there were a subset that did not have PK, and another subset that did not have DNA samples – both were required to remain in the analysis. Second, the bulk of PK data was from the VBP15-004 clinical trial, but this trial sought to reduce burden on patients and families and collected only a single 2 hr post-doc PK sample. This low sampling volume leads to large error bars in the PK calculations of drug exposure and drug clearance. Thus, while this subaim show largely negative results, it was the first such PGx study in DMD and a manuscript has been drafted.

Specific Aim 2. Expand upon published population pharmacokinetics (popPK) models in DMD participants treated with vamorolone to include pharmacogenomics loci defined in Aim 1C.

All data is in hand to complete this aim. This will be completed in Year 2.

4. Impact

- Our validation of two genetic modifier loci in the context of a clinical trial is important and impactful. This points to the importance of these genes in disease pathogenesis and clinical severity.

- It will be important to determine if these same modifiers (or others) have an effect on therapeutic response (efficacy and/or safety). This will be a focus of Year 2.

5. Changes/Problems

No changes in the research plan are anticipated.

We did have a problem where the genotype (SNP chip) data from Mt Sinai showed incorrect duplication of 7 samples. Fortunately, we were able to determine who the duplicated samples were by cross-referencing the SNP data in the DMD gene to the patient's deletion mutations. This permitted both the ID of the duplicated samples, and the ID of the missing samples. This was determined to be an error of the Mt Sinai laboratory, and

they re-genotyped the 7 missing samples. Thus, we were able to diagnose, navigate, and correct this laboratory error.

6. Products

- The genotyping for 2 million DNA polymorphisms in the participants of the vamorolone clinical trials has been completed. Other laboratories can ask for specific genotypes (candidates) from this data set as an approach to genetic modifiers of motor function and drug response.

- A draft of a manuscript on the pharmacogenomics analyses has been completed, and will be submitted during Year 2. Dr. Bill Jusko and his lab are taking the lead on this.

7. Participants & Other Collaborating Organizations

State University of New York at Buffalo: William J Jusko, Xianan Li

University of Padova Italy: Luca Bello, Elena Pegoraro

Carleton University: Utkarsh Dang

8. Special Reporting Requirements

None

9. Appendices

None

STATEMENT OF WORK – 07/31/2023
START DATE - 08/01/2022

Site 1:	Binghamton University – State University of New York	Site 2:	State University of New York at Buffalo	Site 3:	Carleton University	Site 4:	University of Padova
	School of Pharmacy and Pharmaceutical Sciences PO Box 6000 Binghamton NY 13902 PI: Eric Hoffman		404 Pharmacy Building Buffalo, NY 14214-8033 PI: Bill Jusko		3312 Health Sciences Building Ottawa, ON Canada K1S 5B6 PI: Utkarsh Dang		VIA GIUSTINIANI, 5 – PADOVA ITALY 35122 PI: Elena Pegoraro

Specific Aim 1: Define genetic modifiers of baseline motor function, treatment response on motor outcomes, and drug exposure (pharmacogenomics) in DMD participants in the vamorolone clinical trials.	**Timeline**	**Site 1 Binghamton** (Dr. Hoffman)	**Site 2 Buffalo** (Dr. Jusko)	**Site 3 Ottawa** (Dr. Dang)	**Site 4 Padova** (Drs. Bello, Pegoraro)
Major Task 1: Establish subcontracts and complete DNA genotyping of patient samples	Months				
Subtask 1: Establish subcontracts (SUNY Buffalo; Carleton University); University of Padova receives funding for participation in this project from Foundation to Eradicate Duchenne, and will collaborate on data analyses, but not receive funds from this grant (as explained in the original application). ***Completed***	1-3	X	X	X	
Subtask 2: Carry out microarray-based genotyping of 2M DNA polymorphisms (Illumina GDA+PGx genotyping) on n=130 DNA samples from DMD patients in the VBP15-002/003/LTE and VBP15-004 clinical trials. ***Completed***	2-6	X			
Subtask 3: Carry out TaqMan targeted genotyping for specific genetic loci that are not contained in the Illumina microarray (see Table 1 in grant). (n=130 samples x 7 gene loci) ***Completed***	2-6	X			
Subtask 4: Assemble all patient-level data in transportable document for all Sites to access and carry out data analyses (PK, motor outcomes, biomarker, anthropomorphic, DNA mutation, SNP genotyping) ***Completed***	5-6	X	X	X	X
Milestone #1: Demonstrate successful completion of full data set to perform data analyses for all specific aims ***Completed***	6	X	X	X	X

Major Task 2: Complete all bioanalytic approaches in Aims 1 and 2 to define genetic modifiers of treatment response (efficacy and safety), drug exposure, and exposure/response modeling.					
Subtask 1: Carry out Aim 1A. Determine if genetic modifiers defined to date are associated with baseline motor function in DMD boys in the vamorolone trials. ***Completed***	12-24	X		X	X
Subtask 2: Aim 1B. Determine if established genetic modifiers are associated with treatment response (efficacy and safety). ***Year 2***	12-24	X		X	X
Subtask 2: Complete Specific Aim 2. Expand upon published population pharmacokinetics (popPK) models in DMD participants treated with vamorolone to include pharmacogenomics loci defined Aim 1C. ***Year 2***	12-24	X	X		
Milestone #2: Complete all grant aims to define genetic modifiers of treatment response in DMD. ***Year 2***	24	X			

Note: The Government reserves the right to request a revised SOW format and/or additional information.

If human subjects are involved in the proposed study, please provide the projected quarterly enrollment in the following table. Please remove if not appropriate for your project.

As all samples are in hand from completed clinical trials, and are de-identified, the proposed research fulfills exemption #4 of HHS guidelines.