AWARD NUMBER: W81XWH-15-1-0355

TITLE: Neural Correlates of the Y Chromosome in Autism: XYY Syndrome as a Genetic Model

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REPORT DATE: NOVEMBER 2018

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
NOVEMBER 2018	FINAL	15 Aug 2015 - 14 AUG 2018

4. TITLE AND SUBTITLE

Neural Correlates of the Y Chromosome in Autism: XYY Syndrome as a Genetic Model

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0355

5c. PROGRAM ELEMENT NUMBER

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Nemours Children's Health System
Wilmington, DE 19803

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

A multimodal MRI, MRS and MEG (magnetoencephalography) design is employed in cohorts of boys with XYY, who are symptomatic for ASD and control cohorts of idiopathic ASD (ASD-I) and typical development (TD). Targeted recruitment for year 1 totaled 30 enrolled. At the point of this report, 25 subjects have been enrolled, 5 were eliminated for not meeting inclusion criteria upon clinical assessment, 19 have completed data acquisition, and 1 is pending imaging completion. Three of the 19 are pending confirmation of ASD diagnosis. Several more are in various stages of recruitment, scheduling, neuropsychological evaluation or imaging. MRI, MRS and MEG examinations have been conducted in the 17 subjects above with confirmed diagnoses (8TD, 8XYY+ASD, 1 ASD-I). QA suggests in general that complete studies have been tolerated and that data quality is good in the majority of cases. Additional steps to remove MEG trials corrupted by excessive head motion are underway to improve further the evaluable data yield. Data analysis is ongoing, priority having been given to standard approaches for MEG (source localization in BESA followed by consensus "peak" picking), MRS (alignment of "on" and "off" spectra, then subtraction and Gaussian modeling of the GABA and Cr resonances in GANNET) and DTI/HARDI. As mentioned above, about 50% of the data tolerated this strategy robustly. The remaining data are undergoing "scrubbing" to eliminate motion-related artifacts and reduce noise, to accommodate comparable analysis – these data are likely evaluable, but are not reported herein. Dependent variable extraction is underway and preliminary data are shown for illustration of feasibility. It is premature to conduct formal statistical analyses. Recruitment, acquisition and analysis is on track and completion is anticipated in the remaining 12 months of this award.

15. SUBJECT TERMS

Autism spectrum disorder, ASD; 47,XYY syndrome (XYY); neuroimaging; MRI; MEG; Comorbid behaviors

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION

(Technical abstract from application): This proposal addresses two topics of great importance to the ASD community: **mechanisms of heterogeneous clinical expression of ASD** and **mechanisms underlying conditions co-occurring with ASD**, including seizures, attention, and anxiety disorders. Autism affects ~1% of the population (4:1 male pre-dominance) and is heterogeneous with regard to etiological/risk factors, pathogenesis, and clinical presentations. Heritability studies have shown that genetic factors are important in ASD, but dissecting out the relationships among genes, imaging biomarkers, and behavioral phenotypes of ASD is confounded by both genetic heterogeneity and the paucity of neurobiological models. These problems can be circumvented by studying genetically defined ASD subgroups such as 47,XYY syndrome (XYY). XYY occurs in ~0.1% of the male population but has been reported in up to 1% of males with ASD. Approximately 33% of males with XYY satisfy diagnostic criteria for ASD (**XYY+ASD**). The behavioral and neuroimaging biomarkers of XYY+ASD identified in our preliminary studies overlap with those described in ASD-I and, similarly, comorbidities (seizures, attention, and anxiety disorders) exhibited in XYY+ASD are representative of those described in ASD-I. In this proposed study, we will examine the behavioral, neurophysiological and neuroimaging markers of ASD, and specifically compare the variance (**heterogeneity**) of these measures in XYY+ASD versus ASD-I. Having established the level at which XYY+ASD confers imaging/phenotypic heterogeneity reduction, the **mechanism** underlying these measures will be probed via neurochemical magnetic resonance spectroscopy of key neurotransmitters and myelin mapping. Clinically meaningful associations between such measures and behavioral ASD phenotypes will be identified.

A multimodal MRI, MRS and MEG (magnetoencephalography) design is employed in cohorts of boys with XYY, who are symptomatic for ASD and control cohorts of idiopathic ASD (ASD-I) and typical development (TD).

2. KEYWORDS

Autism spectrum disorder, ASD
47,XYY syndrome (XYY)
Neuroimaging
MRI
GABA
MEG
Comorbid behaviors

3. ACCOMPLISHMENTS

What were the major goals of the project?

Major Task 1: Regulatory review and approval processes for studies involving human subjects at 2 sites (CHOP and Nemours/DuPont).

Major Task 2: Recruitment

Major Task 3: Data Acquisition

Major Task 4: Data Analysis

What was accomplished under these goals?

Major Task 1: Regulatory review and approval processes for studies involving human subjects at 2 sites (CHOP and Nemours/DuPont Hospital for Children).

All study protocols have been submitted reviewed and approved by both local IRB's (CHOP / Nemours/DuPont Hospital for Children) as well as central DoD ethics review. Furthermore, since the co-PI Dr. Judith Ross moved to Nemours/DuPont Hospital for Children prior to commencement of this grant funding, approval was obtained by Nemours/DuPont Hospital for Children and all materials were reviewed by DoD. Where appropriate continuing renewal submissions have been submitted and approved in a timely fashion.

Major Task 2: Recruitment

Recruitment for the study totaled 75 enrolled. 4 were eliminated for not meeting inclusion criteria upon clinical assessment, while there were 13 XYY subjects not meeting clinical ASD criteria. While these are, by inclusion criteria, not consistent with the goals of the written grant proposal, we found them to be a highly relevant control group and have indeed included them in the protocol (under separate funding) and labeled as XYY-ASD.

Group	N
TD	20
ASD-I (idiopathic)	20
XYY+ASD	18
XYY-ASD	13
Excluded (based on psych.)	4

Sample demographics and neuropsychological assessment data is provided below:

	Age (yrs) ± S.D.	ADOS CSS ± S.D. (ASD measure)	CELF CLI ± S.D. (language measure)	DAS NonVerbal Cluster ± S.D. (general cognitive measure)
TD	12.9±3.3	0.9±0.4	103.9±12.9	112.5±15.0
ASD-I	13.9±3.4	6.5±1.8 *	98.0±17.5	103.9±15.7
XYY+ASD	12.9±3.5	6.6±2.1 ’	78.8±12.9 ‘	90.9±15.4 ‘
XYY-ASD	12.8±3.6	2.1±1.6 ’	90.6±16.4 ‘	96.2±14.2 ‘
	No significant differences	‘p<0.05 TD vs XYY *p<0.05 TD vs ASD-I	‘p<0.05 TD vs XYY	‘p<0.05 TD vs XYY

As can be seen from the clinical and neuropsychological assessment profiles, the XYY+ASD group share features with the ASD-I group (e.g. ADOS CSS), while the XYY group who do not meet clinical ASD diagnostic criteria nonetheless show atypical assessment values (lying between TD and XYY+ASD on most measures).

Major Task 3: Data Acquisition

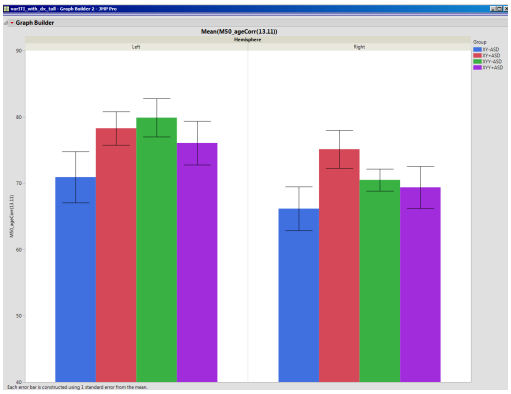
MRI, MRS and MEG examinations have been conducted in the 71 subjects above with confirmed diagnoses (20TD, 18XYY+ASD, 20 ASD-I, 13XYY-ASD). QA continues to suggest that imaging protocols have been tolerated and that data quality is good in the majority of cases. A variety of approaches have been tailored to increase evaluable data yield, via the removal of artifacts such as excessive head motion during MEG or DTI acquisitions and frequency drift during MR spectroscopy experiments.

Major Task 4: Data Analysis

To describe the brain correlates of XYY syndrome, and to place them in context with findings in “idiopathic” ASD, we employed a multimodal battery of imaging, neurochemistry and electrophysiological assays which have demonstrated utility in prior studies of ASD and related disorders. Specifically we examine the components of the auditory evoked response to simple tone stimuli as well as “oddball” paradigms involving vowel contrasts. Data is collected using MEG (as opposed to EEG) to provide superior artifact rejection and localization ability. Subsequently we employ diffusion weighted MRI to assess the white matter microstructure of the auditory system and major language pathways (superior longitudinal fasciculus) as well as spectrally edited “MEGAPRESS” magnetic resonance spectroscopy, to estimate GABA. For each measure most analyses center on linear mixed models (LMM’s) with subject as a random effect. Fixed effects include Group (TD, ASD-I, XYY+ASD, XYY-ASD), Hemisphere and, where appropriate, stimulus token with age as a covariate. Interactions with hemisphere in particular prompt post-hoc analyses separately by hemisphere.

Preliminary Analysis and Trend Observation of Dependent Variables acquired under Major Task 3 and Analyzed in Major Task 4:

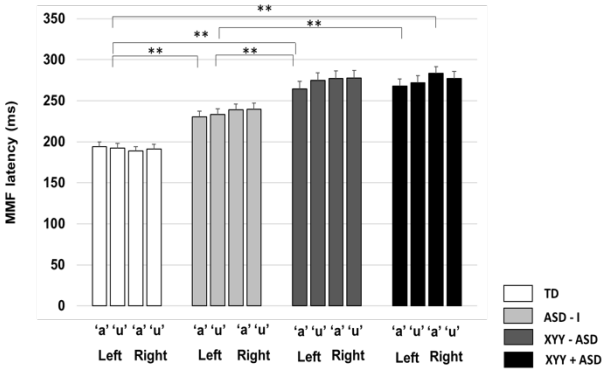
1. M50 Evoked Response Latency



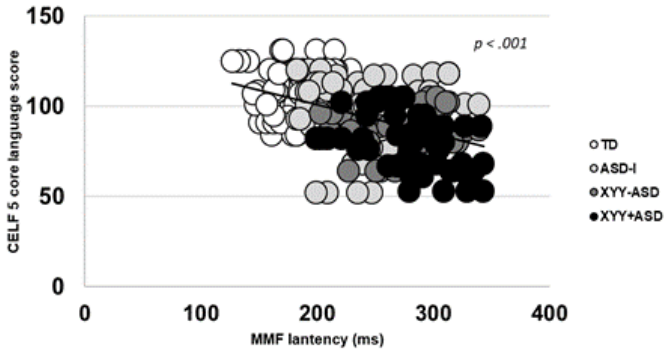
The ~50ms cortical component of the auditory evoked response to 500Hz sinusoidal stimuli exhibits a 6.9ms delay in XYY (Figure to right, green and purple bars) vs TD (blue bars) (trend p=0.1), analogous to M50 latency prolongation seen in idiopathic ASD (red bars). M50 latency prolongation relative to TD is exhibited bilaterally. Of note, both XYY+ASD but also XYY-ASD (not meeting ASD diagnostic criteria) nonetheless exhibit latency prolongation (with no differences between XYY+ASD and XYY-ASD), suggesting a mechanism that might be observed in idiopathic ASD but that is invoked by the Y chromosome duplication itself, independent of a clinical ASD diagnosis. There are no significant differences between XYY+ASD vs ASD-I

2. MMF "mismatch field" Latency

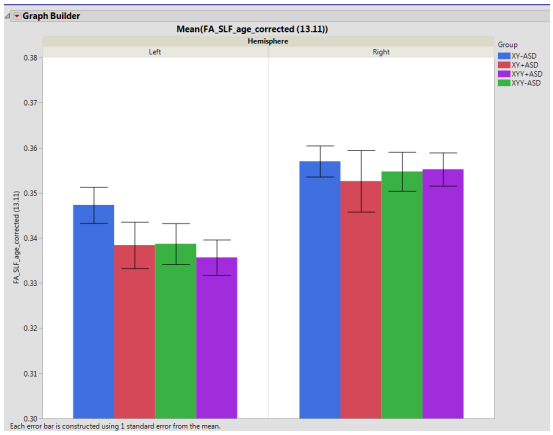
To understand the neurophysiological mechanisms underlying auditory language discrimination processing of vowel stimuli in children / adolescents with XYY syndrome, MEG measured mismatch fields (MMFs) arising from left and right superior temporal gyrus (STG) during an auditory oddball paradigm with vowel stimuli (/a/ and /u/) in children / adolescents with XYY with and without ASD, idiopathic ASD (ASD-I) or typically developing (TD).



Linear Mixed Model (LMM) analysis (fixed effects – Group, Hemisphere and Token with age) revealed a significant main effect of group on MMF latency ($p < .001$, Figure to left), with no main effect of Hemisphere ($p > .15$) or Token ($p > .66$) and no interaction ($p > .95$). Delayed MMF latencies were found in XYY with and without ASD group, or ASD-I groups compared to the TD group ($p's < .001$, Figure left). Both XYY with and without ASD groups showed delayed MMF latency compared to the ASD–I group ($p < .001$). However, similar to the M50 results above, there were no group differences between XYY with ASD and XYY without ASD ($p > 0.05$). Consistent with previous reports, delayed MMF latency was negatively correlated with lower language ability of CELF - 5 core language scores (r=-0.46, p<.001, Figure), indicating that delayed MMF latency predicts poor behavioral language function.

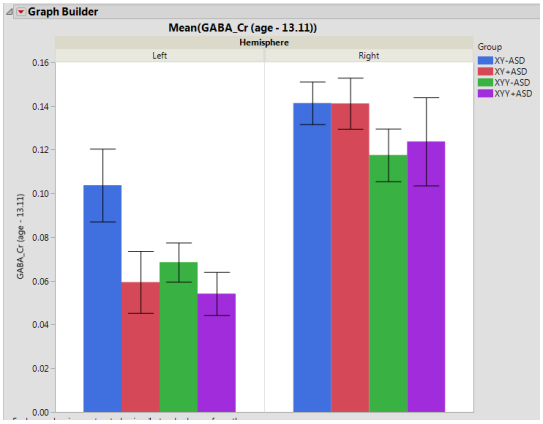


3. **Diffusion weighted imaging of the distal auditory pathway (subserving Heschl's gyrus) and superior longitudinal fasciculus**



Left hemisphere (Figure to left, leftmost bars) diffusion fractional anisotropy (white matter maturation) suggests immature WM (lower FA values, $p<0.05$) in both ASD-I (red) and XYY (green, purple) cohorts compared to TD controls (blue). Interestingly, the right hemisphere (Figure to left, rightmost bars) data (elevated with respect to corresponding left hemisphere values, perhaps because of more complete maturation) shows no group differences (perhaps attributable to plateauing maturation in all groups). Further longitudinal studies are warranted to explore hemisphere white matter maturational trajectories and differences thereof.

4. **Spectrally edited MEGAPRESS magnetic resonance spectroscopy**



Estimation of the critical inhibitory neurotransmitter GABA is achieved via localized spectrally-edited MEGAPRESS spectroscopy, optimized for suppression of co-edited macromolecules (incorporating RF pulses at 1.9 and 1.5ppm – symmetric about 1.7ppm). GABA estimation is performed manually using HLSVD in jMRUI and expressed relative to the metabolite creatine (Cr) to compensate for session, subject and location differences affecting signal.

Significant deficit in GABA/Cr is observed in XYY (green, purple) compared to TD (blue, $p<0.01$), driven especially by left hemispheric levels (leftmost bars). Again, analogous to the diffusion findings of #3 above, it is of note that right hemisphere values (rightmost bars) are in general greater than the corresponding left hemisphere, suggesting early and more complete maturation in the right hemisphere. Correspondences between the two sets of barcharts (diffusion and GABA) suggest maturational delays in both microstructure and neural circuitry (proxied by neurotransmitter levels) are most clearly depicted in the left hemisphere in this age range. A slight difference between the two sets of charts is exhibited in the right hemisphere GABA levels which are nonetheless reduced compared to TD or ASD-I (red) in both XYY groups, perhaps indicating a lower plateau level, or perhaps indicating yet incomplete maturation.

Table 2. Co-morbidities associated with XYY+ASD

Medical and Psychiatric diagnoses or clinically significant findings	XYY+ASD (n=8)	TD (n=8)	Childhood Prevalence	Prevalence in ASD-I
FEATURES				
Hypotonia	7 (88%)	0		51%
Tremor	2 (25%)	0	0.1 – 22%	
MEDICAL DIAGNOSES				
Motor Delay/Dyspraxia	6 (75%)	0	< 5%	34%
Seizures	0	0	1%	14-35%
PSYCHIATRIC DIAGNOSES				
ADD or ADHD	5 (63%)	0	2 – 16%	28%
Verbal or Motor Tic	2 (25%)	0	5 – 10%	9%
Oppositional Defiant	4 (50%)	0	1 – 16%	30%
Depression	2 (33%)	0	<1%	1.4%
Anxiety	6 (75%)	0	15 – 20%	42%
Bipolar/mood disorder	1 (13%)	0	0.4 – 6.3%	26%

Table 2. XYY is associated with significant occurrence of co-morbid behaviors/diagnoses. Although stratified analyses is premature, a later goal of this study is to identify neural features relating to such behaviors

Additional analyses are exploring the rates of identification of co-morbid diagnoses in XYY, wherein both motor and psychiatric anomalies are prevalent.

Summary of findings

Across measures spanning neuroimaging, neurochemistry and neurophysiology, subjects in the XYY cohorts exhibit analogous observations to ASD-I, namely delayed evoked responses, immature white matter and deficient levels of GABA compared to age-matched typically developing peers. Several of these phenomena exhibit hemispheric differences in ability to distinguish groups, with left hemisphere measures most sensitively identifying group differences (in this age-range). Since these measures have recognized developmental trajectories (which also may differ between hemispheres) the findings in XYY may be hypothesized to reflect developmental immaturity compared to age-matched TD peers.

Across these measures, there were no resolvable differences between subject with 47,XYY syndrome with and without ASD diagnoses. This could be argued as supporting the contention that the imaging, chemistry and neurophysiological signatures are sensitive to the genomic character of participants (i.e. relating to the Y chromosome aneuploidy). Alternatively, it could be argued that the subjects with XYY who do not meet clinical ASD diagnostic criteria nevertheless have atypical clinical and behavioral assessments. This latter hypothesis is somewhat challenged by the absence of even a trend towards graduated imaging anomalies in XYY-ASD vs XYY+ASD.

Addressing the question of whether ASD associated with XYY (XYY+ASD) is representative of ASD-I in general, the concordance between imaging findings in XYY

subjects and ASD-I relative to TD provides a level of support, at least at the level of the brain scanning measures.

Taken together, we conclude that from an imaging, neurochemistry and electrophysiological standpoint, XYY can be considered as a relevant human genetic model of features of broader idiopathic ASD.

What opportunities for training and professional development has the project provided?

Pursuant to his involvement in this study, Dr Luke Bloy, an expert in imaging methodology, has become well-versed in clinical and behavioral sequelae of imaging observation in both idiopathic ASD and related genetic syndromes. He is embarking on a research career where these two trains of endeavor and inquiry dovetail.

How were the results disseminated to communities of interest?

Initial analysis were presented at the recent International Meeting for Autism Research (*Structural and Functional Characteristics of XYY - Relationship to ASD*, *L. Bloy et al, IMFAR, 2017*). An abstract describing the radiologic structural findings in XYY has been submitted to the 2019 ASNR annual meeting (noting anomalies of the corpus callosum – both thinning and thickening as the most conspicuous findings).

Additionally two papers are currently submitted and under review/in revision. The first, titled "*Auditory Evoked Response Delays in Children with 47,XYY syndrome*" presents the M50 and M100 findings. The second, a case report titled "*NLGN4Y copy number variations and neurocognitive/autism phenotypes: Behavioral Phenotype In Male With XYY and absent NLGN4Y Expression"* discusses the behavioral and neuroimaging phenotype of an individual with 47,XYY who is missing the NLGN4Y gene and who exhibits a broadly "typical" profile of imaging and electrophsyiological measures (as distinct from the pattern observed in the remainder of the XYY cohort, even those without an ASD diagnosis).

An additional manuscript detailing the mismatch field findings is in the final stages of preparation for submission to Autism Research. Work is underway to develop a manuscript describing the GABA observations.

What do you plan to do during the next reporting period to accomplish the goals?

This is the final report. Nonetheless we are continuing to explore correlations between the above observations and clinical and behavioral assessments as well as to conduct a full multi-modal statistical analysis. These will likely lead to a further summary manuscript.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project? - Two areas of impact can be identified: firstly, the recognition that, at least in terms of clinical/behavioral and imaging/electrophysiologic measures, XYY as a syndrome appears to share many of the features of idiopathic ASD and thus aneuploidy of the Y chromosome might well be a promising candidate human genetic model for ASD research in general. Secondly, it appears that brain imaging, neurochemistry and electrophysiological assays, although motivated by observations in idiopathic ASD, may well be more tightly coupled to underlying genetic anomalies (e.g. XYY) than to behavioral phenotype. At least for microstructural diffusion findings and electrophysiological latency measures this conclusion appears consistent with a recent set of studies of another genetic factor (also identified as increasing risk for – but not certainty of – ASD) – CNV of 16p11.2. Perhaps these "genetics first" approaches will continue to inform the field of imaging correlates of shared pathways.

What was the impact on other disciplines? - Embedded in above answer.

What was the impact on technology transfer? - There is no IP that has been developed under this award.

What was the impact on society beyond science and technology? - Identifying commonalities at the level of the brain between idiopathic ASD and boys with 47,XYY syndrome might be expected to lead to optimized (building on lessons learned in ASD treatment) behavioral interventions (and ultimately perhaps pharmaceutical approaches) to mitigate the behavioral sequelae of XYY and thus improve outcomes and quality of life (QOL).

5. CHANGES/PROBLEMS

Changes in approach and reasons for change - none

Actual or anticipated problems or delays and actions or plans to resolve them

This study is now concluded. Manuscripts are in review, preparation and planning.

Changes that had a significant impact on expenditures – none

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents – none

6. PRODUCTS

Publications, conference papers, and presentations – see above – 2 abstracts, 3 manuscripts, with several manuscripts underway.

Website(s) or other Internet site(s) – none

Technologies or techniques – none

Inventions, patent applications, and/or licenses – none

Other Products – none

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

No change: Roberts, Miller, Bloy, Ross

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Study is now concluded

What other organizations were involved as partners?

Nemours / Dupont Hospital for Children

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: None