PHARMACOVIGILANCE OF NEUROPSYCHIATRIC ADVERSE REACTIONS TO MEFLOQUINE

by
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ABSTRACT

Introduction

Mefloquine (MQ) is a synthetic quinoline derivative antimalarial drug that is structurally related to quinine. Recently, MQ has been the subject of regulatory scrutiny, as awareness has grown of the potential for adverse neuropsychiatric reactions associated with the drug’s use. Mild neuropsychiatric adverse reactions to MQ prophylaxis may predict the development of more serious neuropsychiatric effects, including neurological disorders, which regulators now warn may occasionally be permanent. Despite the potential utility of prodromal reactions in predicting more serious reactions, it is unclear which specific reactions should prompt discontinuation. Prodromal and more serious neuropsychiatric reactions to MQ may also mimic other conditions, and the absence of knowledge of a distinct syndrome of neuropsychiatric adverse reactions associated with the drug’s use may limit specificity of case finding in pharmacogenetic studies.

Methods

A review of international drug safety guidance was conducted with the aim of defining common categories of neuropsychiatric adverse reactions to MQ, including those that may be prodromal. Next, latent class analysis (LCA) of reported categories of neuropsychiatric adverse reactions to antimalarial drugs, including MQ, was performed with the aim of identifying a distinct neuropsychiatric syndrome class associated with the drug’s use. Lastly, a pharmacogenetic study of adverse outcomes associated with use of
MQ was conducted with the aim of assessing the utility of surrogate neuropsychiatric phenotypes in genetic association studies.

Results

There is broad international agreement that certain reactions to MQ, including anxiety, depression, restlessness, confusion, sleep disturbances, and certain neurological disorders should prompt discontinuation of the drug. In LCA of reported reactions, certain of these, including confusion, define a distinct syndrome class strongly associated with MQ use. Use of non-specific surrogates for this syndrome, including posttraumatic stress disorder, may result in a lack of sufficient power to detect associations in pharmacogenetic studies of reactions to MQ.

Conclusions

Pharmacovigilance of neuropsychiatric adverse reactions to MQ may be improved through recognition of prodromal symptoms, and appreciation of the distinct syndrome of neuropsychiatric adverse reactions to the drug. The identification of improved diagnostic indicators for this syndrome may enable more powerful studies of predictors of serious adverse reactions to MQ use.
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CHAPTER 1

PHARMACOVIGILANCE OF NEUROPSYCHIATRIC ADVERSE REACTIONS TO MEfloquINE: AN INTRODUCTION

1.1. Introduction

The World Health Organization (WHO) defines pharmacovigilance as “the science and activities relating to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems” (World Health Organization, 2006). This dissertation presents the results of a number of studies intended to better detect, assess, understand, and prevent neuropsychiatric adverse reactions associated with exposure to the synthetic quinoline derivative antimalarial mefloquine (MQ) (commonly marketed as Lariam).

MQ was originally developed during a Vietnam War era drug discovery effort that was sponsored and organized by the U.S. military (Tigertt, 1969). First synthesized in 1969 (Ohnmacht, Patel, & Lutz, 1971), MQ emerged as a favored compound of this effort and was first licensed internationally in the 1980s (Gutteridge, 1989). However, by the time of its U.S. licensing in 1989, increased attention focused in the medical and scientific literature on the potential for adverse neuropsychiatric reactions to the drug during both prophylactic and therapeutic use (World Health Organization, 1989a, 1989b, 1989c). Over the subsequent quarter century, this attention has led to increased regulatory scrutiny, including recent pharmacovigilance studies in the U.S. which culminated in the addition of a boxed warning to the approved U.S. MQ labeling, describing a risk of
lasting and potentially disabling psychiatric and neurological effects from the drug (U.S. Food and Drug Administration, 2013).

Despite such efforts, the pharmacoepidemiology of these more serious neuropsychiatric adverse reactions to MQ and the molecular mechanisms underlying these reactions remain poorly characterized. Specific risk factors including genetic risk factors that may predict the development of these more serious neuropsychiatric adverse reactions to MQ also remain poorly characterized. Only the development of seemingly mild prodromal neuropsychiatric symptoms — such as sleep disturbances and changes in mood — during early use of the drug have been identified by drug regulators as a clinically significant risk factor that may inform guidance for safer use of the drug in practice (European Medicines Agency, 2014a, 2014b, 2014c). Unfortunately, neuropsychiatric symptoms consistent with such prodromal reactions may be independently common in overseas settings (Nevin, 2015b) or in patient populations such as military personnel and civilian travellers in which the drug is typically used (Nevin, Pietrusiak, & Caci, 2008), or may be reported as a result of nocebo effect. Even when causally attributable to the drug, the occurrence or reporting of such prodromal symptoms may be subject to biased ascertainment (Rønn, Rønne-Rasmussen, Gøtzsche, & Bygbjerg, 1998), or be attributed to other causes (Schlagenhauf & Steffen, 2000). In the absence of better emphasis of the clinical significance of such prodromal symptoms, and of more concerted efforts at their valid and reliable identification, this may potentially limit the utility of such guidance in preventing more serious adverse effects.

Additionally, emphasis in the literature only on single symptoms — rather than combinations of neurologic and psychiatric reactions — associated with drug use may
further limit the utility of any such guidance. While U.S. regulators have acknowledged that more serious neurologic reactions to MQ are typically accompanied by lasting psychiatric symptoms (Levin, 2013; U.S. Food and Drug Administration, 2013), particular combinations of these reactions are not well described in the literature as being associated with MQ use. The absence in the literature of a well-defined syndrome of neuropsychiatric reactions specifically associated with MQ use may limit case finding, particularly of more serious neuropsychiatric reactions to the drug in population-based studies, and thus may consequently limit further studies into the pharmacoepidemiology and pharmacogenetics of these neuropsychiatric reactions.

In the absence of an accepted syndrome definition, when such combinations of reactions do occur, they may potentially mimic other neuropsychiatric disorders or confound their diagnosis (Livezey, Oliver, & Cantilena, 2016; Nevin & Ritchie, 2015; Nevin, 2015a). Particularly in retrospective studies, appropriate indicator diagnoses may be of potential utility in identifying those in whom serious neuropsychiatric adverse reactions to MQ may have been overlooked.

1.2. Specific Aims, Objectives, and Conceptual Framework

This dissertation will explore three specific aims related to improving the pharmacovigilance of neuropsychiatric adverse reactions to MQ.

Aim 1 will seek to better identify and classify those common neuropsychiatric reactions or symptoms associated with MQ use, and to identify and classify those potentially prodromal reactions that may predict more serious neuropsychiatric adverse reactions to the drug. This aim will be explored in a study of international drug labeling,
whose objectives will include identifying those reactions — and categories of reaction as
defined by the Medical Dictionary for Regulatory Activities (MedDRA) — listed as
necessitating physician consultation or drug discontinuation and for which there is
international agreement, and identifying the degree of such international agreement. This
aim may serve to inform improvements in drug labeling safety guidance with the
pharmacovigilance goal of better preventing more serious neuropsychiatric adverse
reactions to the drug.

Aim 2 will seek to identify a neuropsychiatric adverse reaction syndrome
associated with the drug’s use, consistent with reports of prodromal and more serious
neuropsychiatric adverse effects. This aim will be explored in a study involving analysis
of reported antimalarial drug adverse event data in the U.S. Food and Drug
Administration (FDA) adverse events reporting system (FAERS) database. This study
will seek to identify and define a measurement model of neuropsychiatric adverse
reactions, and through latent class analysis, determine the syndrome class prevalence
among FAERS reports, and its association with antimalarial drugs including MQ
commonly used among travellers. This aim may serve to inform improvements in case
finding, with the pharmacovigilance goals of better detecting and assessing these more
serious adverse reactions.

Aim 3 will then explore the feasibility of investigating genotype-phenotype
associations in retrospective population-based pharmacogenetic studies of MQ, in order
to ultimately identify genetic risk factors that may predict the development of
neuropsychiatric adverse reactions. This aim will be explored in a candidate gene study
of a large U.S. military cohort prescribed MQ, in which various neuropsychiatric
outcomes are used as surrogates for the neuropsychiatric adverse reaction syndrome, evaluating the hypothesis that these are associated with polymorphisms in candidate gene ABCB1. This chapter’s methods may serve as a foundation for future candidate gene studies employing stored genetic material and archived data available through the U.S. military. This aim may serve to improve the future evaluation of candidate gene associations with appropriate indicator diagnoses, with the pharmacovigilance goal of better understanding the molecular origins of neuropsychiatric adverse reactions.

A figure illustrating the conceptual framework underlying these aims and objectives is included as Figure 1.1.

1.3. Organization of the Dissertation

This introductory chapter serves as brief background, introduction, and motivation for the substantive aims of the dissertation, with a detailed discussion of the history of the drug’s use and rising awareness of its potential chronic neuropsychiatric effects left to existing recent publications on the subject (Nevin & Croft, 2016; Nevin & Ritchie, 2015; Nevin, 2015a; Ritchie, Block, & Nevin, 2013). The following three chapters present Aims 1—3 in order. Each of these three chapters is presented as a manuscript either submitted or in preparation for submission to peer-reviewed journals. The final chapter summarizes and discusses the work presented in the dissertation, and concludes with recommendations for future research and pharmacovigilance activities.
Figure 1.1. Conceptual Framework of the Dissertation
CHAPTER 2

NEUROPSYCHIATRIC ADVERSE REACTIONS TO MEFLOQUINE: A SYSTEMATIC COMPARISON OF PRESCRIBING AND PATIENT SAFETY GUIDANCE IN THE US, UK, IRELAND, AUSTRALIA, NEW ZEALAND, AND CANADA*

Abstract

The antimalarial drug mefloquine (MQ) is associated with neuropsychiatric adverse reactions, some of which may predict the development of more serious effects. Although prescribing guidance in the United States drug label (DL) recommends that MQ be discontinued (DC) at the onset of neuropsychiatric symptoms, only certain reactions are listed in both the DL and the corresponding patient medication guide (MG) with a recommendation to DC or to consult a physician should they occur. To identify possible prodromal reactions for which there is complete or partial agreement in prescribing and patient recommendations, a systematic comparison of international drug safety labeling was performed. The full text of each DL and MG (or equivalent) from 6 primarily English-speaking countries were reviewed to identify specific reactions with corresponding recommendations in drug safety labeling. Percentage agreement across the countries in corresponding recommendations was determined by MedDRA highest-level grouping term (HLGT). Recommendations were found for reactions in 22 neuropsychiatric HLGTs. Complete or partial international agreement was found for reactions in 11 (50%) HLGTs, including sleep disorders and disturbances. This analysis

suggests opportunities for physicians to improve patient counseling and for international
drug regulators to clarify language in MQ safety labeling to reflect national risk-benefit
consideration.

2.1. Introduction

Mefloquine (MQ) is a synthetic quinoline-derivative antimalarial drug structurally
related to quinine that exhibits idiosyncratic central nervous system toxicity (Nevin, 2014). In double blinded studies, a range of neuropsychiatric adverse reactions—including strange or vivid dreams, dizziness, vertigo, concentration impairment, anxiety, and depression—are reported by 29-77% of MQ users at prophylactic doses of 250 mg weekly (Overbosch et al., 2001; Schlagenhauf et al., 2003). According to a retrospective cohort study, among those reporting adverse reactions to MQ, 21% of those reporting nightmares and 33% of those reporting cognitive dysfunction identified these as persisting for over 3 years after use (Ringqvist, Bech, Glenthøj, & Petersen, 2015).

A boxed warning added to the United States (US) drug label (DL) in 2013 notes that MQ may cause “neuropsychiatric adverse reactions that can persist after mefloquine has been discontinued”. Prescribing guidance in the US DL now recommends to discontinue (DC) MQ at the onset of neuropsychiatric symptoms, as certain of these may suggest an individual risk of “more serious psychiatric disturbances or neurologic adverse reactions” that could occur with continued use of the drug. The US DL now cautions that psychiatric reactions “ranging from anxiety, paranoia, and depression to hallucinations and psychotic behavior can occur with mefloquine use” and “have been reported to continue for months or years after mefloquine has been stopped”. The US DL now also
cautions that certain neurological reactions may persist or become permanent (U.S. Food and Drug Administration, 2013).

The highly prescriptive safety guidance in the current DL reflects its gradual evolution over the prior quarter century. At the time of the US licensing of MQ in 1989, the original DL instructed physicians only that, “[d]uring prophylactic use, if signs of unexplained anxiety, depression, restlessness or confusion are noticed, these may be considered prodromal to a more serious event. In these cases, the drug must be discontinued” (F. Hoffman-La Roche, 1989). This language was subtly updated in 2002, changing the previously exclusive list of prodromal reactions to an illustrative list by stating, “if psychiatric symptoms such as acute anxiety, depression, restlessness or confusion occur, these may be considered prodromal to a more serious event. In these cases, the drug must be discontinued and an alternative medication should be substituted” (F. Hoffman-La Roche, 2002).

The 2002 DL update introduced potential ambiguity as to whether US physicians were to counsel patients that the onset of any psychiatric symptom should be considered prodromal and prompt DC, or merely that those explicitly listed and similar reactions should be considered prodromal and prompt DC. The 2013 DL update further clarified that “[d]uring prophylactic use, the occurrence of psychiatric symptoms such as acute anxiety, depression, restlessness or confusion suggest a risk for more serious psychiatric disturbances or neurologic adverse reactions [emphasis added]. In these cases, the drug should be discontinued and an alternative medication should be substituted”. The boxed warning addressed any remaining potential ambiguity in this illustrative list by emphasizing that if a patient develops any neurologic or psychiatric symptoms while
taking MQ, the drug should be discontinued and an alternative medication should be substituted (U.S. Food and Drug Administration, 2013).

The reasons for these changes were not made explicitly clear in the 2013 DL, nor in the accompanying US Food and Drug Administration (FDA) drug safety communication (U.S. Food and Drug Administration, 2013). However, the following year in a 2014 pharmacovigilance review, the European Medicines Agency (EMA) concluded “a causal relationship between mefloquine and the occurrence of long lasting and even persistent neuropsychiatric effects”, and noted a “strong suspicion” that MQ could in some cases cause “permanent brain damage” (European Medicines Agency, 2014a). The EMA noted that no “specific risk factors” could be identified for these effects, and concluded, “[f]or that reason, only the advice – to stop taking mefloquine if neuropsychiatric reactions or changes to their mental state occur – can be given as a precautionary measure” (European Medicines Agency, 2014a).

In the US, in the case of certain drugs “that pose a serious and significant public health concern”, the FDA may require certain specific safety guidance be communicated directly to patients in the form of a medication guide (MG) provided at the time of dispensing, which complements counseling received by the patient at the time of prescribing (U.S. Food and Drug Administration, 1998). The FDA requires a MG when it determines patient adherence to directions for use are considered crucial to a drug’s effectiveness, when the drug has serious risks relative to benefits, or when patient safety guidance in the MG could help prevent “serious adverse effects” (U.S. Food and Drug Administration, 1998). Consistent with this final rationale, the MG for MQ was first required by the FDA in 2003 (F. Hoffman-La Roche, 2003).
The current US MG explicitly lists certain psychiatric or neurologic adverse reactions for which patients are recommended to consult with a physician (CP) or healthcare provider prior to taking their next dose. Although the US boxed warning clearly recommends to DC “if psychiatric or neurologic symptoms occur”, as is the case with the MG, only certain specific neurologic or psychiatric adverse reactions are explicitly listed in the current US DL with a recommendation to DC.

The rationale for the specific choice of listed adverse reactions for which the US MG and DL are in correspondence in recommending to DC or CP is not clear. The choice may reflect consensus decision-making between the FDA and the drug’s manufacturers on those prodromal or precursor adverse reactions that “suggest a risk for more serious psychiatric disturbances or neurologic adverse reactions” and which should therefore be specifically highlighted in light of risk-benefit considerations particular to the US regulatory and legal environment. Such decision-making may include a consideration of the predictive value of a particular prodromal or precursor adverse reaction in foretelling more serious reactions with continued use of the drug.

In other countries, based on the results of independent regulatory decision-making and potentially differing risk-benefit considerations, the particular choice of listed neurologic and psychiatric adverse reactions for which DC or CP may be recommended may differ from those of the US. International agreement in specific listed MQ neurologic and psychiatric adverse reactions for which safety guidance recommends DC or CP may therefore reflect agreement on the strength of the evidence and the consistency of risk-benefit decision-making motivating those recommendations.
To compare and contrast current international recommendations for actions to be taken in response to specific MQ neurologic and psychiatric adverse reactions, and to identify those categories of adverse reactions for which there is international agreement in listing such recommendations, a systematic comparison was performed of current prescribing and patient safety guidance in the US and five other primarily English-speaking countries: the United Kingdom (UK), Ireland (IRL), Australia (AUS), New Zealand (NZ), and Canada (CAN).

2.2. Methods

2.2.1. Prescribing and Patient Safety Documents

Prescribing safety guidance in the UK and IRL is provided in a document referred to as a Summary of Product Characteristics; in AUS, as a Product Information; in New Zealand (NZ), as a Data Sheet; and in CAN, as a Product Monograph — all of which are referred herein as a DL. Although not always comparably mandated in each country, patient safety guidance similar to that provided in the US MG is provided in AUS and NZ in a document referred to as Consumer Medicine Information; in IRL, as the Patient Leaflet; in the UK, as the Package Leaflet; and in CAN, as Information for the Patient — all of which are referred herein as a MG.

The most recent MQ DL and MG as of December 2015 were identified through a search of the websites of national drug regulators and drug manufacturers in each of the six countries. The DL and MG were retrieved for the innovator product (marketed as Lariam) in those countries where the innovator product remained marketed, and for the generic product in those countries where the innovator product had been withdrawn.
2.2.2. Review of Prescribing and Patient Guidance

The full text of each DL and MG was then reviewed by two clinicians to identify recommendations for actions to be taken in response to specific listed neurologic or psychiatric adverse reactions. Disagreements between the clinicians during review were resolved by consensus.

Where a recommendation stated the drug “should” or “must” be discontinued or stopped at the onset of a listed adverse reaction, this was categorized as a recommendation to DC. Where the text did not include an explicit direction to DC or suggestion that it “may be necessary to stop”, but only included a recommendation to “consult immediately” or “consult” a healthcare provider at the onset of a listed adverse reaction, this was categorized as a recommendation to CP. Where contradictory guidance for a listed adverse reaction appeared in different locations in the text, a recommendation to DC took precedence.

Where one or more adverse reactions were listed ambiguously within a paragraph that contained a particular recommendation associated with a smaller list of adverse reactions or a more general description of an adverse reaction, the recommendation was deemed to apply to that term. For example, based on the following paragraph in the US DL, a recommendation to DC was deemed to apply to the adverse reactions of dizziness, vertigo, tinnitus, and loss of balance: “Neurologic symptoms such as dizziness or vertigo, tinnitus, and loss of balance have been reported. These adverse reactions may occur early in the course of mefloquine use and in some cases have been reported to continue for months or years after mefloquine has been stopped. Dizziness or vertigo, tinnitus, and loss of balance have been reported to be permanent in some cases. During prophylactic
use, if neurologic symptoms occur, the drug should be discontinued and an alternative medication should be substituted”.

Where the text referred explicitly only to an adverse reaction occurring in the context of a pre-existing condition or contraindication, this was not included in analysis. Similarly, if an adverse reaction was described only in a section of the text containing guidance applicable prior to starting MQ, without referencing explicitly that the adverse reaction could also be caused by MQ, it was not included in analysis. Likewise, if an adverse reaction appeared only in a table, without an explicit inclusive reference in the text to a specific recommendation, it was also not included in analysis.

Adverse reactions were considered as neurologic or psychiatric according to the Medical Dictionary for Regulatory Activities (MedDRA) (Brown, Wood, & Wood, 1999), version 18.1, if the MedDRA lowest-level term (LLT) matching the adverse reaction was categorized within either the MedDRA nervous system or psychiatric disorders system organ class (SOC). For example, the LLT dizziness, categorized in the MedDRA under the SOCs cardiac disorders, vascular disorders, and nervous system disorders, was considered neurologic for the purposes of this analysis. Where a listed LLT could be considered both psychiatric and neurologic, it was considered psychiatric. For example, the LLT insomnia, categorized in the MedDRA under the SOCs psychiatric disorders and nervous system disorders, was considered psychiatric. Where a particular adverse reaction — such as when expressed in consumer language — did not explicitly match an LLT, the closest relevant lexical or conceptual variant to MedDRA terminology was substituted. For example, the MedDRA LLT “masked facies” was substituted for the adverse reaction “difficulties with facial expression”. Similarly the MedDRA LLT
“restless” was substituted for the adverse reaction “feeling restless”. As similar or equivalent adverse reactions may be reported by a range of terminology, all LLTs were grouped according to their MedDRA preferred term (PT). For example, “restlessness” and “feeling restless” were grouped together on the basis of their common PT “restlessness”.

2.2.3. International Agreement in Corresponding Prescribing and Patient Guidance

Adverse reactions were organized according to their MedDRA high level group term (HLGT). Those countries whose MG and DL both included a recommendation either to DC or CP at the onset of one or more PT were deemed to be in correspondence for that adverse reaction. Countries in correspondence for one or more PT within each HLGT were identified, and for each HLGT, the percentage agreement across all 6 countries of corresponding DL and MG recommendations to DC or CP for one or more PT was determined. HLGTs for which corresponding MG and DL recommendations to DC or CP for one or more PT were in agreement for all 6 countries were deemed to be in complete international agreement, while HLGTs for which corresponding MG and DL recommendations to DC or CP for one or more PT were in agreement for 2 to 5 countries were deemed to be in partial international agreement.

2.3. Results

At the time of analysis, the drug remained licensed in all 6 countries, although the innovator product had been withdrawn from the US as of 2011 (Department of Health and Human Services, 2011) and from CAN as of 2013. The most recent generic US DL and MG available were dated June 2013, and the most recent generic CAN DL and MG
available were each dated March 2011. Lariam-branded DL and MG were available for the UK, IRL, AUS and NZ. The UK DL was dated December 2015 and the MG was dated April 2015. The IRL DL was dated June 2015 and the MG was dated May 2015. The AUS DL and MG were both dated November 2014, while the NZ DL and MG were both dated August 2014.

In addition to being explicitly recommended by the US DL, the DLs of the UK and IRL also explicitly recommended DC at the onset of general neurologic or psychiatric symptoms. Echoing the language in the earlier EMA document (European Medicines Agency, 2014a), the UK and IRL DL recommended “If neuropsychiatric reactions or changes to the mental state occur during mefloquine chemoprophylaxis [emphasis added], the patient should be advised to stop taking mefloquine and seek medical advice immediately so that mefloquine can be replaced by alternative malaria prevention medication”.

Among the 6 DL and 6 MG, additional patient and prescriber guidance was identified for specific adverse reactions within 22 distinct neuropsychiatric HLGTs, comprising 14 psychiatric HLGTs as shown in Table 2.1, and 8 neurologic HLGTs as shown in Table 2.2. There was complete agreement across all 6 countries in corresponding MG and DL recommendations to DC or CP for adverse reactions within 4 (18%) of the HLGTs. These HLGTs were anxiety disorders and symptoms, changes in physical activity, depressed mood disorders and disturbances, and deliria (including confusion). There was partial agreement across 3 of the 6 countries (US, UK, and IRL) in corresponding MG and DL recommendations to DC or CP for adverse reactions within 3 (14%) additional HLGTs. These HLGTs were disturbances in thinking and perception,
personality disorders and disturbances in behavior, and suicidal and self-injurious behaviors NEC. There was also partial agreement across 2 of the 6 countries (UK and IRL) in corresponding MG and DL recommendations to DC or CP for adverse reactions within 4 (18%) additional HLGTs. These were neuromuscular disorders, schizophrenia and other psychotic disorders, sleep disorders and disturbances, and peripheral neuropathies.

In a single country (US), there was also corresponding MG and DL guidance to DC or CP for adverse reaction within 2 (9%) additional HLGTs. These were cranial nerve disorders (excluding neoplasms) and neurological disorders NEC. Among adverse reactions within 9 (41%) additional HLGTs, although patient guidance in the MG from at least 2 countries each recommended CP, there was no corresponding guidance provided to physicians for these specific adverse reactions in the DL, as shown in Table 2.3.

2.4. Discussion

2.4.1. Agreement in Prescribing and Patient Guidance

This analysis finds complete international agreement across the 6 countries in corresponding prescribing and patient safety guidance to CP or DC in response to specific psychiatric adverse reactions to MQ within 4 HLGTs. These include the common adverse reactions of depression and anxiety within the HLGTs depressed mood disorders and disturbances and anxiety disorders and symptoms. The DL of the UK, IRL, AUS, and NZ each describe anxiety and depression as occurring in ≥1/100-1/10 of prophylactic users.
In contrast, this analysis finds only partial international agreement across two or more countries in corresponding prescribing and patient safety guidance to CP or DC in response to specific neurologic and psychiatric adverse reactions within an additional 7 HLGTs. These include the very common adverse reaction of abnormal dreams within the HLGT sleep disorders and disturbances. The DL of the UK, IRL, AUS, and NZ each describe abnormal dreams as occurring in ≥1/10 of prophylactic users. Intriguingly, this analysis finds a recommendation to DC at the onset of reactions within the HLGT sleep disorders and disturbances only in the DL and MG of the UK and IRL. In contrast, the MG in CAN provides no recommendation for such reactions and notes only that certain of these usually “do not cause people to stop taking the medicine”. In further contrast, the MG of three other countries, the US, AUS, and NZ, explicitly recommend only CP for such reactions. Unusually, the NZ MG lists abnormal or strange dreams as serious side effects requiring immediate CP. However, in contradiction to the DL that lists abnormal dreams as “very common”, the MG states that such serious side effects are “rare”.

For those other adverse reactions among the remaining 6 HLGTs for which there is also partial international agreement in corresponding prescribing and patient safety guidance, the DLs of the UK, IRL, AUS, and NZ each list their incidence as unknown, or do not specifically list the adverse reactions. In contrast, for the adverse reactions vertigo and dizziness, which are listed among the remaining 2 HLGTs cranial nerve disorders and neurological disorders NEC respectively, there is corresponding prescribing and patient safety guidance only in the DL and MG of a single country (US). The DL of the UK, IRL, AUS, and NZ each describe vertigo and dizziness as common, occurring in ≥1/100-1/10 of prophylactic users.
2.4.2. Relevance of Findings

Amidst stated declining demand and market share, the innovator, Roche, has elected to withdraw Lariam-branded MQ from the market in a number of additional countries besides the US and CAN, including IRL, Germany, and Denmark (Arznei-Telegramm, 2016a). Although there are concerns the drug may soon be withdrawn from other countries for similar reasons (Hawkes, 2016), MQ is likely to remain available internationally in generic forms for some time. This analysis provides important insights that may be relevant during patient counseling and in the consideration of potential future improvements to MQ drug label safety guidance for the drug’s use in prophylaxis.

For example, as MQ prophylaxis is commonly prescribed for travelers, who by definition may be far from the prescribing physician or healthcare provider at the time that adverse reactions occur, CP may not be immediately feasible, delaying any potential recommendation to DC in the case of a particular psychiatric or neurologic adverse reaction for which the MG recommends only CP. Similarly, even in the event that CP is immediately available — such as by telephone or email — lack of explicit recommendation in a MG for a patient to seek CP for a particular adverse reaction may also delay any potential physician direction to DC should the patient not recognize its significance. A review of narrative reports of MQ adverse reactions may aid national drug regulators in determining whether failed recognition of the significance of a particular adverse reaction identified in this analysis, or delays in CP related to travel, may have contributed to more serious adverse reactions. Such a review may also aid regulators in determining whether these serious adverse reactions may have been
potentially preventable by a more explicit recommendation to DC, such as through specific listing in the prescribing and patient guidance documents.

Current national guidelines for use of MQ in malaria prophylaxis among travelers may be informed by current safety guidance in the national DL and MG. Differences in prescribing and patient safety guidance between countries may inform differential national recommendations for use of MQ, and contribute to disagreement in the setting of common international guidelines. This analysis may aid as a starting point for developing consensus for international guidelines on the use of MQ, in spite of significant international disagreement in current safety guidance.

Similarly, disagreement in international prescribing and patient safety guidance may contribute to contradictory situations where similar patients from similar countries, who experience identical neurologic or psychiatric adverse reactions from MQ while traveling, are directed to take discordant actions in response. For example, under current guidance in country-specific MG, travelers from the UK and IRL are directed to DC MQ at the onset of abnormal dreams, while travelers from AUS and NZ are directed merely to CP and not to DC at their onset. In contrast, travelers from the US are provided with no specific advice for actions to be taken at the onset of this very common adverse reaction, while travelers from CAN are advised that bad dreams are “usually mild” and “do not cause people to stop taking the medicine”. Such discordance, while presumably reflecting differing national risk-benefit decision-making, may contribute to confusion among travelers and to country-specific guidance not being followed. This analysis is expected to aid in clearly identifying those classes of neurologic and psychiatric adverse reactions
for which there is disagreement in international recommendations, permitting physicians and healthcare providers to appropriately emphasize national guidance.

2.4.3. Limitations

This analysis has a number of limitations. Significantly, this analysis is limited to a review of prescribing and patient safety guidance from only 6 developed countries. These countries were chosen based on their principal use of English and a shared common cultural and linguistic heritage. The measures of international agreement in prescribing and patient safety guidance from this analysis can therefore not be generalized to other countries, whose prescribing and patient guidance was not specifically reviewed.

Additionally, this analysis relies on a subjective interpretation of the language in the various DL and MG. Although reflecting consensus opinion among the study authors, based on the imprecise nature of this language and the systematic but arbitrary rules employed in this analysis, there may be reasonable disagreement by others as to whether a particular DL or MG should be interpreted as recommending DC or CP at the onset of a particular adverse reaction. This limitation reflects the disagreement that may be expected between individual prescribers and patients in interpreting recommendations in the DL and MG.

In certain cases, this analysis may also have assigned certain adverse reactions to a HLG distinct from what might have been seemingly implied by the patient or prescribing guidance. For example, based on the use of the MedDRA to categorize particular listed adverse reactions, this analysis assigned many neurologic LLTs that might be considered most consistent with peripheral neuropathy — including pain and numbness — to the HLG neurological disorders NEC. However, both the UK and the
IRL DL state, “[m]efloquine should be discontinued in patients experiencing symptoms of neuropathy, including pain, burning, tingling, numbness, and/or weakness in order to prevent the development of an irreversible condition [emphasis added]”. This limitation reflects the fact that idiomatic language commonly used by patients, physicians, and other healthcare providers may not necessarily adhere to the standard MedDRA vocabulary (Inácio, Airaksinen, & Cavaco, 2015).

2.5. Conclusions

MQ prescribing guidance in a growing number of countries now recommends DC at the onset of any neuropsychiatric adverse reaction. This analysis has identified certain common neuropsychiatric adverse reactions to MQ for which DC is specifically recommended and for which there is complete or partial international agreement the reaction be explicitly listed in both prescribing and patient guidance.

The results of this analysis suggest opportunities for physicians in these countries to improve patient counseling by emphasizing the need to DC at the onset of these adverse reactions. The results of this analysis also suggest opportunities for international drug regulators to clarify language in future updates to remaining MQ DL and MG to better reflect national risk-benefit considerations for continued use of the drug.
Table 2.1. Psychiatric Adverse Reactions to Mefloquine, Prescribing and Patient Guidance, by Country

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<th>Adverse reaction HLGT and LLT</th>
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Notes: a. LLT thinking irrational, b. LLT thinking abnormal, c. LLT mood change, d. LLT abnormal behavior, e. LLT mental state abnormal, f. LLT sleep disorder, g. LLT suicidal ideation, h. LLT self-injurious behavior, i. "Usually mild and do not cause people to stop taking the medicine".

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<tr>
<th>Adverse reaction HLGT and LLT</th>
<th>US DL</th>
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Table 2.3. Country-Specific Mefloquine Patient and Prescribing Guidance, Number Recommending to DC or to CP for One or More Adverse Reactions, and Number and Countries with Corresponding Guidance for Both, by Adverse Reaction HLGT.

<table>
<thead>
<tr>
<th>Adverse reaction HLGT</th>
<th>MG n</th>
<th>DL n</th>
<th>Corresponding MG and DL guidance n (%)</th>
<th>Countries</th>
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<tr>
<td>Anxiety disorders and symptoms</td>
<td>6</td>
<td>6</td>
<td>6 (100)</td>
<td>US UK IRL AUS NZ CAN</td>
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<tr>
<td>Changes in physical activity</td>
<td>6</td>
<td>6</td>
<td>6 (100)</td>
<td>US UK IRL AUS NZ CAN</td>
</tr>
<tr>
<td>Depressed mood disorders and disturbances</td>
<td>6</td>
<td>6</td>
<td>6 (100)</td>
<td>US UK IRL AUS NZ CAN</td>
</tr>
<tr>
<td>Deliria (including confusion)</td>
<td>6</td>
<td>6</td>
<td>6 (100)</td>
<td>US UK IRL AUS NZ CAN</td>
</tr>
<tr>
<td>Disturbances in thinking and perception</td>
<td>5</td>
<td>3</td>
<td>3 (50)</td>
<td>US UK IRL</td>
</tr>
<tr>
<td>Personality disorders and disturbances in behavior</td>
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<td>3</td>
<td>3 (50)</td>
<td>US UK IRL</td>
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<tr>
<td>Suicidal and self-injurious behaviors NEC</td>
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<td>3</td>
<td>3 (50)</td>
<td>US UK IRL</td>
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<td>2 (33)</td>
<td>UK IRL</td>
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<tr>
<td>Schizophrenia and other psychotic disorders</td>
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<td>3</td>
<td>2 (33)</td>
<td>UK IRL</td>
</tr>
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<td>Sleep disorders and disturbances</td>
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<td>2 (33)</td>
<td>UK IRL</td>
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<td>2</td>
<td>2 (33)</td>
<td>UK IRL</td>
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<tr>
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<td>1</td>
<td>1 (17)</td>
<td>US</td>
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<td>1</td>
<td>1 (17)</td>
<td>US</td>
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<tr>
<td>Mood disorder and disturbances NEC</td>
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<td>0 (0)</td>
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<td>Headaches</td>
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<tr>
<td>Movement disorders (including parkinsonism)</td>
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<td>Psychiatric and behavioral symptoms NEC</td>
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<td>Cognitive and attention disorders and disturbances</td>
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<td>Psychiatric disorders NEC</td>
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<td>Seizures (including subtypes)</td>
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<td>0</td>
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</table>

Abbreviations: US—United States, UK—United Kingdom, IRL—Ireland, AUS—Australia, NZ—New Zealand, CAN—Canada, DL—Drug Label (or national equivalent), MG—Medication Guide (or national equivalent), DC—discontinue drug, CP—consult physician, HLGT—MedDRA high level group term, NEC—not elsewhere classified.
CHAPTER 3
NEUROPSYCHIATRIC ADVERSE REACTIONS TO MEFLOQUINE:
LATENT CLASS ANALYSIS OF FDA ADVERSE EVENT REPORTING SYSTEM
DATA

Abstract
Mefloquine (MQ) use is associated with risk of neuropsychiatric adverse reactions, but particular combinations of neurologic or psychiatric reactions that may be associated with MQ use are not well described in the literature. This study sought to identify whether a distinct neuropsychiatric syndrome could be identified associated with MQ use from reports of adverse events. Latent class analysis of U.S. Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) data was performed using indicators defined by Medical Dictionary for Regulatory Activities (MedDRA) neurologic and psychiatric high level group terms (HLGTs), in a study dataset of FAERS reports (N=5,332) of reactions to common antimalarial drugs. A distinct neuropsychiatric class was identified that was strongly and significantly associated with reports of MQ use (OR=3.92, 95% CI 2.91-5.28) and defined by a high probability of symptoms of deliria including confusion and disorientation. This syndrome is consistent with early descriptions of toxic encephalopathy from MQ case reports at the time of the drug’s initial licensing. Identification of the distinct set of clinical features of this neuropsychiatric syndrome may aid in improved case finding in pharmacoepidemiological and pharmacogenetic studies of more serious adverse reactions to the drug.
3.1. Introduction

Use of mefloquine (MQ), a synthetic quinoline derivative antimalarial, is associated with risk of neuropsychiatric adverse reactions. Certain of these may be considered prodromal (Nevin & Byrd, 2016) and predict the development of more serious adverse effects, including psychiatric and neurological symptoms that regulators warn may persist after MQ has been discontinued (European Medicines Agency, 2014a; U.S. Food and Drug Administration, 2013).

Prodromal reactions to MQ, which include very common symptoms of sleep disturbance and abnormal dreams and common symptoms of anxiety and depression, (Nevin & Byrd, 2016), may be independently common in settings such as international travel (Schlagenhauf & Steffen, 2000) and military operations (Nevin, 2015b) where MQ may be used. Prodromal reactions may also be subject to over-reporting, or may be reported due to nocebo effects (Clift & Grabowski, 1996). Even when causally attributable to the drug, as evidenced by case reports (Livezey et al., 2016; Nevin, 2012a; Peterson, Seegmiller, & Schindler, 2011), individual prodromal symptoms may risk being attributed to causes other than to MQ, reducing the effectiveness of guidance that the drug be discontinued at their onset.

Certain individual symptoms consistent with those of the MQ prodrome may also occur from other drugs in settings of MQ use. For example, two other antimalarial drugs commonly used— for prophylaxis of chloroquine (CQ)-resistant malaria — atovaquone-proguanil (AP) and doxycycline (DX) — are also associated with symptoms of sleep disturbance and abnormal dreams (Nevin & Croft, 2016), although these are reported less commonly than with MQ (Ohrt et al., 1997; Overbosch et al., 2001; Wallace
et al., 1996). Similarly, CQ has also long been associated with symptoms similar to those caused by MQ (Boudreau et al., 1993; Lobel et al., 1991), although as with AP and DX, their occurrence with use of CQ is not accompanied by warnings to discontinue the drug at their onset. Owing to the perceived ubiquity of such symptoms in settings of MQ use, the unique guidance applicable to MQ to discontinue the drug at the onset of prodromal symptoms may risk being minimized or overlooked.

While U.S. regulators at the Food and Drug Administration (FDA) have acknowledged that more serious neurologic reactions to MQ are typically accompanied by lasting psychiatric symptoms (Levin, 2013; U.S. Food and Drug Administration, 2013), particular combinations of neurologic and psychiatric reactions associated with MQ use are not well described in the literature, and the prevalence of these particular combinations are also not well described. The absence in the literature of a well-characterized and specific syndrome of neuropsychiatric adverse reaction associated with MQ use may risk such combinations of reactions confounding the diagnosis of other neuropsychiatric conditions (Nevin & Ritchie, 2015), which may also limit pharmacoepidemiological and pharmacogenetic studies of such adverse reactions.

This study therefore sought to determine whether there is a distinct neuropsychiatric syndrome class associated with MQ use, and if so, to identify specific clinical features strongly associated with the syndrome that might inform improvements in case finding.
3.2. Methods

Latent class analysis (LCA) is a statistical method that enables the grouping of individuals into one or more distinct classes on the basis of responses to a finite number of indicators, such as the presence or absence of various reported symptoms (Garrett & Zeger, 2000). LCA is a commonly accepted method for improving accuracy in psychiatric diagnosis in the absence of an objective “gold standard” (Faraone & Tsuang, 1994). Although the reporting of drug adverse reactions may involve selecting from potentially thousands of distinct medical terms, these may be categorized into a manageable number by means of the Medical Dictionary for Regulatory Activities (MedDRA) vocabulary, which translates reported reactions into standard terminology known as lowest level terms (LLT), and groups these into medically similar or equivalent preferred terms (PT) (Brown et al., 1999). The MedDRA further categorizes PTs multiaxially into one or more of 26 top level system organ classes (SOC), and within each SOC, to typically one of a few dozen distinct high level group terms (HLGT) (Brown et al., 1999), thus making the presence or absence of reactions categorized at SOC- or HLG T-level of potential utility as indicators in LCA.

In this analysis, both SOC- and HLG T-level indicators were used in conducting case-level LCA among drug adverse events reported to the FDA. Reports utilized in this analysis included those for the three antimalarial drugs commonly used in prophylaxis of CQ-resistant malaria — MQ, AP, and DX; and as controls, those for CQ and the anti-motility agent loperamide (LP), a common co-exposure in settings where antimalarials are used (Steffen, 1991).
Descriptive statistical analyses were conducted using *STATA Version 14* (StataCorp, College Station, TX), and LCA was conducted using *MPLUS Version 7.4* (Muthen & Muthen, Los Angeles, CA).

### 3.2.1. Study Data

Reports of adverse reactions are maintained by the FDA in the FDA Adverse Event Reporting System (FAERS) (Moore, Cohen, & Furberg, 2007). Study data were obtained by querying the *Qscan FDA* database (DrugLogic, Reston, VA) — a commercial aggregator of publicly-available FAERS data — for all FAERS reports listing MQ, AP, DX, CQ, and LP or their common generic and trade variants as suspect or non-suspect drugs. At the time of the query, the database was current through the end of 2015. Duplicate reports based on same age, sex, and FDA receipt dates were automatically excluded from the query.

### 3.2.2. MedDRA Categorization of FAERS Report Reactions

Where reactions in historical FAERS reports did not match a current MedDRA PT, these were updated to the current MedDRA vocabulary if these matched a current LLT, or where there was no match, manually to a lexical variant of a current LLT. Where a PT was multiaxial, PT assignment was prioritized to the SOC psychiatric disorders and nervous system disorders, in that order. Categorized reactions of a general, social, or procedural nature (i.e. SOCs general disorders and administration site conditions; investigations; injury, poisonings, and procedural complications; surgical and medical procedures; and social circumstances) were excluded. Reactions describing congenital and perinatal conditions (i.e. SOCs congenital, familial and genetic disorders; and pregnancy, puerperium and perinatal conditions), neoplastic conditions (i.e. SOC
neoplasms benign, malignant and unspecified including cysts and polyps), immune and infectious disorders (i.e. SOCs immune system disorders; infections and infestations), and conditions of the reproductive system (i.e. SOC reproductive system and breast disorders) were also excluded.

For each FAERS report, binary indicators corresponding to the 15 remaining SOCs, and to individual HLGTs within the psychiatric and nervous system disorder SOCs, were created on the basis of reported PTs.

3.2.3. Study Datasets

By drug, datasets were created collapsing by case number any positive response in binary SOC- and HLGT-level indicators across original and subsequent FAERS reports. By case, the minimum of reported age (when not unknown) was assigned as case age; sex was assigned as male, female or unknown; and for the three primary study drugs MQ, AP, and DX, indication was assigned as either for malaria prophylaxis or other/unknown, with malaria prophylaxis taking precedence when there was conflicting data across subsequent FAERS reports. To exclude neonatal cases potentially due to maternal exposure, cases less than 1 year of age were excluded.Datasets by drug were then pooled, and to ensure independence in subsequent LCA, cases appearing in multiple study drug datasets were excluded. Two study datasets were created from the pooled dataset: the primary dataset consisting of MQ, AP, and DX cases; and a control dataset consisting of CQ and LP cases.

3.2.4. Latent Class Indicator Selection

In order to select neuropsychiatric HLGT-level indicators for subsequent LCA, initial latent class models were estimated from the primary dataset using binary SOC-
level indicators with greater than 2% overall prevalence. Two separate models were estimated to test for convergent results: the first, a fully conditionally dependent (full) model; and the second, a partially conditionally dependent (partial) model. For the partial model, these were iteratively fit, specifying conditional dependence between SOC-level indicator pairs with significant pairwise bivariate residuals (Pearson $\chi^2 > 1.96$), until a final model could be estimated with non-significant pairwise bivariate residuals. Among each model, fit statistics were compared, with final model selection made on the basis of Bayesian information criteria (BIC) (Garrett & Zeger, 2000). The partial model was evaluated for evidence of residual conditional dependence by means of multiple random probability-weighted pseudoclass assignment, whereby SOC-level indicators with Bonferroni-adjusted significant p-values from pairwise logistic regression in 5 or more of 100 draws by pseudoclass were deemed to exhibit significant conditional dependence.

From each selected model, neuropsychiatric classes were then subjectively identified on the basis of relatively elevated conditional probabilities for psychiatric and nervous system disorder SOC-level indicators. Among cases with most likely membership in the identified neuropsychiatric classes, neuropsychiatric HLGT-level indicators were similarly evaluated for evidence of differential measurement by means of multiple random probability-weighted pseudoclass assignment. Indicators with Bonferroni-adjusted significant p-values from logistic regression by drug in 5 or more of 100 draws by pseudoclass were deemed to exhibit significant differential measurement. HLGT-level indicators free of evidence of significant differential measurement across model neuropsychiatric classes, and with greater than 2% overall prevalence, were considered for selection for the final model.
3.2.5. HLGT-Level Latent Class Analysis

Latent class models with 2 through 4 classes were estimated from the primary dataset from the selected neuropsychiatric HLGT-level indicators, permitting pairwise conditional dependence between all indicators, with final model selection made on the basis of BIC. Association of HLGT-level class with drug was assessed by manual 3-step testing in MPLUS (Asparouhov & Muthén, 2013), according to published methods (Vermunt, 2010) with results reported as odds ratios (OR) and 95% confidence intervals (95% CI).

3.3. Results

The number of cases by drug and final dataset are shown in Figure 3.1. There were 974 MQ, 469 AP, 4,018 DX, 443 CQ, and 6,808 LP cases identified in the original data. Respectively, 10, 5, 3, 3, and 17 cases were excluded for being under age 1. When pooled, 196 of the 12,674 cases were excluded for listing multiple study drugs. Of the remaining 12,478 cases, 933 were MQ, 450 AP, and 3,949 were DX and comprised the primary dataset consisting of 5,332 cases. The control dataset of 7,146 cases consisted of 422 remaining CQ cases and 6,724 remaining LP cases. The sex and age group of study cases by drug are shown in Table 3.1. The age and sex distribution of cases varied significantly by drug in both the primary dataset ($\chi^2=84.7$, $p<0.001$ for age group; $\chi^2=77.3$, $p<0.001$ for sex) and the pooled data overall ($\chi^2=356.0$, $p<0.001$ for age group; $\chi^2=222.5$, $p<0.001$ for sex). In the primary dataset, the distribution of reported indications also varied significantly by drug ($\chi^2>1000$, $p<0.001$), as shown in Table 3.2.
Total numbers and proportions of cases with reported reactions in each of the 15 study SOCs were tabulated by drug in the primary dataset and are shown in Table 3.3. The proportions of reported reactions varied significantly by drug across all SOCs (all \( p<0.05 \)). By drug, the most commonly reported reactions were within the psychiatric disorders SOC for MQ (56.5% of cases, most commonly anxiety), the nervous system disorders SOC for AP (29.1% of cases, most commonly headache), and the gastrointestinal disorders SOC for DX (31.4% of cases, most commonly nausea).

Reactions were reported in fewer than 2% of cases overall within 2 SOCs — ear and labyrinth disorders, and endocrine disorders — which were excluded from subsequent LCA.

In the primary dataset, reactions within the nervous system disorder SOC were reported for 20 HLGTs at frequencies ranging from 0.03% to 22.2%, and within the psychiatric disorder SOC for 23 HLGTs at frequencies ranging from 1.1% to 12.6%.

In the SOC-level LCA, partial models with 2 through 7 classes had BIC of 54006, 53994, 53967, 53937, and 53940, respectively; and full models with 2 through 5 classes had BIC of 54213, 54162, 54158, and 54193, respectively. The 6 class partial and 4 class full models were selected based on the best fit by BIC. Class labels were assigned subjectively on the basis of conditional probabilities, with a mild and moderate neuropsychiatric class identified in each, as shown in Figures 3.2 and 3.3.

The partial model had entropy of 0.560 and a non-significant overall univariate residual (\( \chi^2=0.003, p>0.05 \)). Despite non-significant pairwise bivariate residuals (\( \chi^2<1.96, p>0.05 \) for all comparisons), the overall bivariate residual was significant (\( \chi^2=13.79, p>0.05 \)), with evidence on subsequent pseudoclass testing of significant
residual conditional dependence between indicator pairs for the respiratory and vascular
SOCs, and for the hepatobiliary and metabolic SOCs in the moderate neuropsychiatric
class; and between indicator pairs for the cardiac and renal SOCs in the multi-system
class. In contrast, the full model had entropy of 0.872, and non-significant overall
univariate and bivariate as well as pairwise bivariate residuals.

Of the 43 neuropsychiatric HLGTs, 13 had evidence of significant differential
measurement across both the mild and moderate neuropsychiatric classes in the full and
partial models. Of the remaining 30, 8 occurred at 2% prevalence or greater and exhibited
no significant differential measurement in the moderate neuropsychiatric class in both the
full and partial models, and these were selected as indicators for the final LCA. Assigning
subjects to their most likely class, prevalence of reported reactions by HLGT in both the
moderate and mild neuropsychiatric classes, in the partial and full models, by most-likely
class and overall without regard to class assignment, are shown in Table 3.4. HLGTs
selected for the final model included communication disorders and disturbances; deliria
(including confusion); dementia and amnestic conditions; depressed mood disorders and
disturbances; neuromuscular disorders; peripheral neuropathies; psychiatric disorders
NEC; and seizures (including subtypes).

In the HLGT-level LCA, models with 2 through 4 classes had BIC of 11908,
11972, and 12038, respectively. The 2 class model was selected, and had entropy of
0.971, and non-significant overall univariate and bivariate as well as pairwise bivariate
residuals. A neuropsychiatric syndrome class with overall prevalence 4.3% was identified
without evidence of significant differential measurement by drug for any HLGT-level
indicator. In contrast the non-syndrome class demonstrated evidence of significant
differential measurement for three HLGT-level indicators: deliria (including confusion), dementia and amnestic conditions, and depressed mood disorders and disturbances. Among cases in the most-likely non-syndrome class, simple logistic regression showed these indicators were significantly more likely to be reported with MQ, and significantly less likely to be reported with DX. Conditional probabilities of HLGT-level indicators for the syndrome and non-syndrome class are reported in Table 3.5.

Based on most-likely class assignment, the prevalence of the syndrome in the primary study dataset was 10.3% with MQ, 2.0% with AP, and 3.5% with DX. On three-step testing, the syndrome class was positively associated with MQ (OR 3.92, 95% CI 2.91-5.28, p<0.001), and negatively associated with AP (OR 0.35, 95% CI 0.14-0.85, p=0.020) and DX (OR 0.38, 95% CI 0.28-0.51, p<0.001). Applying the fitted model to the control dataset, based on most-likely class assignment, the prevalence of the syndrome was 3.3% with CQ and 6.0% with LP. On three-step testing there was no significant association of the syndrome with CQ (OR=0.46, 95% CI 0.17-1.28, p=0.138) or with LP (OR=2.172, 95% CI 0.78-6.04, p=0.138). In contrast, across the pooled dataset, the association was negatively associated with LP (OR=0.73, 95% CI 0.59-0.91, p=0.005), was insignificant for AP (OR=0.45, 95% CI 0.18-1.09, p=0.075), DX (OR=0.83, 95% CI 0.65-1.06, p=0.134), and CQ (OR=0.341, 95% CI 0.12-1.01, p=0.05), and remained positive and significant for MQ (OR 3.97, 95% CI 3.08-5.12, p<0.001).

On post-hoc analysis of the study dataset, assigning cases to their most likely class, among the 5,095 cases assigned to the most-likely non-syndrome class, the five most commonly reported HLGTs across all 15 study SOCs were gastrointestinal signs and symptoms; neurological disorders NEC; epidermal and dermal conditions;
respiratory disorders NEC; and anxiety disorders and symptoms. In contrast, among the 237 cases assigned to the most-likely syndrome class, the five most commonly reported HLGTs were deliria (including confusion); neurological disorders NEC; anxiety disorders and symptoms; sleep disorders and disturbances; and depressed mood disorders and disturbances. The prevalence of these most commonly reported syndrome HLGTs together with the most commonly reported reactions within each (with greater than 5% overall prevalence), by most-likely class, are listed in Table 3.6, along with odds ratios for reporting each HLGT in the most-likely syndrome vs. non-syndrome class.

Among the study dataset, among cases assigned to the most-likely class, the proportion of syndrome cases by drug did not vary significantly by age group (<18 years, 18-65 years, and 65+ years) for MQ (p=0.292 by chi-square), AP (p=0.073), or DX (p=0.151). In contrast, while the proportion of syndrome cases by drug did not vary significantly by sex for AP (p=0.184), they did vary significantly by sex for MQ (p=0.001) and DX (p=0.002), being strongly associated in each with male sex by simple logistic regression (OR 2.15, 95% CI 1.37-3.38, and OR 1.70, 95% CI 1.20-2.41, respectively).

The proportion of syndrome cases by drug also varied significantly by reported indication for MQ (p<0.001), being strongly associated with malaria prophylaxis (OR 2.29, 95% CI 1.50-3.51) by most-likely syndrome class assignment — an association not seen with AP (p=0.318) or DX (p=0.426 by Fischer’s exact test). Overall, based on most-likely class assignment, the prevalence of the syndrome class in the primary study dataset among cases with a reported indication of malaria prophylaxis was 16.0% with MQ, 2.5% with AP, and 0% with DX.
3.4. Discussion

This analysis has found among reports of adverse events associated with common antimalarial drugs evidence of a distinct neuropsychiatric syndrome class that is strongly associated with MQ use. This class is defined by a very high probability (82.7%) of symptoms of deliria, including confusion and disorientation, and a moderate probability of other serious psychiatric and neurologic symptoms including dementia and amnesia conditions (18.6%), and seizures (18.1%). While not exclusive to MQ, among reports of adverse events from drugs used for prophylaxis of CQ-resistant malaria, this syndrome is nearly exclusive to use of MQ.

Intriguingly, the characteristic features of this syndrome, including confusion, amnesia, and seizures, were among the earliest reported serious adverse neuropsychiatric effects of MQ noted in the literature following the drug’s initial European licensing in the mid-1980s. Initial case reports described a syndrome of acute brain or toxic encephalopathy, typically with confusion and seizure (Bernard et al., 1987, 1989; Rouveix et al., 1989). Isolated amnesia was also reported (Lapras, Vighetto, Trillet, & Garin, 1989), as was psychosis associated with symptoms of confusion (Björkman, 1989). An early case series, which emphasized neurological reactions, also noted that difficulties with concentration were a common feature (Patchen, Campbell, & Williams, 1989). Unlike other possibly more subjective neuropsychiatric symptoms, these severe neuropsychiatric reactions, suggestive of organic CNS dysfunction, would be expected to have been subject to minimal reporting bias and nocebo effect.

In this study, among reports of adverse events, this syndrome is commonly accompanied by symptoms that are considered prodromal, including insomnia, abnormal
dreams, anxiety, and depression (Nevin & Byrd, 2016), and neurological symptoms such as dizziness, vertigo, and paresthesias. While this study’s methods could not determine whether these reported symptoms preceded the more serious characteristic symptoms of this syndrome, such a progression would be consistent with case reports (Livezey et al., 2016; Nevin, 2012a; Peterson et al., 2011) and regulatory warnings describing such symptoms as prodromal to more serious events.

Interestingly, this study found that this syndrome is more common among reports of adverse events from male users than female users of MQ. This is in contrast to other published findings, in which milder neuropsychiatric symptoms, some of which might be considered prodromal, are more commonly reported among female users of the drug (van Riemsdijk et al., 2004; van Riemsdijk, Sturkenboom, Pepplinkhuizen, & Stricker, 2005). While the reasons for this are unclear, it is plausible that this finding could reflect a greater propensity among certain males, such as military personnel, to fail to discontinue MQ at the onset of prodromal symptoms (Boudreau et al., 1993), consequently risking the development of more serious neuropsychiatric effects (Nevin & Byrd, 2016).

3.4.1. Limitations

This study has a number of important limitations that require the results to be interpreted with caution. This study relied on reported drug adverse event data within the FAERS database. This data is known to suffer from significantly incomplete and delayed reporting (Getz, Stergiopoulos, & Kaitin, 2012; Ma, Marinovic, & Karaca-Mandic, 2015), and the accuracy of this data, including important demographic and suspect drug elements, is often questionable (Getz et al., 2012). Partially to address the potential for suspect drug misattribution, this study included reports in the analysis even when the
drug was listed as non-suspect. This had the benefit of increasing sample size for the LCA, but possibly as a consequence, there were a high number of cases of the neuropsychiatric syndrome associated with DX. In contrast, in post-hoc analysis of the study dataset, based on most likely class assignment, there were no syndrome cases among those in whom DX was reported as indicated for malaria prophylaxis. The high prevalence of this delirium-like syndrome among non-prophylaxis DX cases may have been due to common use of this drug among elderly patients for non-malaria indications, for whom adverse event reports may have been filed primarily for non-study suspect drugs.

Perhaps most significantly, this study relied on a novel application of LCA to the analysis of FAERS data. The use of SOC- and HLGT-level indicators, derived from reported adverse reactions that would not be expected to be independent of each other and which may be potentially subject to significant measurement error, including over- and under-reporting by drug, required the use of fully conditionally dependent models, and resulted in a final 2 class HLGT-level model that demonstrated significant differential measurement in the non-syndrome class. The potential threats to model validity encountered in this analysis may limit the broader application of the LCA approach for analysis of FAERS data.

Despite these limitations, these results are nonetheless intriguing, and suggest that case finding for more serious neuropsychiatric reactions to MQ may be improved through the identification of specific reactions that are highly specific to the identified syndrome class. In this respect, it is noteworthy that all but 16 of the 197 cases (8.1%) in the study dataset reporting one or more reactions within the HLGT that included confusional state,
disorientation, and delirium, were assigned by the model to the most-likely syndrome class, and that this association was very strong and significant (OR=1039.2, 95% CI 581.1-1855.4, p<0.001). These results suggest that among those users reporting adverse neuropsychiatric reactions to MQ, AP, and DX, such reactions should be considered nearly pathognomonic for this syndrome.

3.5. Conclusion

Early reports of adverse effects from MQ described a syndrome characterized by confusion, amnesia, and seizure. This analysis has found evidence of this syndrome among a large number of subsequent reports of adverse events from common antimalarial drugs within the FAERS database. While this syndrome does not appear to be unique to reports of adverse reactions to MQ, it is strongly and significantly associated with MQ use, particularly in prophylaxis, and is not significantly associated with reports from other common antimalarial drugs used in the prophylaxis and treatment of CQ-resistant malaria. Although MQ may cause additional neuropsychiatric effects including prodromal symptoms, the clinical identification of the more specific symptoms of this syndrome may aid in improving pharmacovigilance, and particularly case finding in pharmacoepidemiology and pharmacogenetic studies of more serious adverse reactions to the drug.
Table 3.1. Sex and Age Group of Study Cases in the Primary and Control Datasets, by Drug

<table>
<thead>
<tr>
<th></th>
<th>Primary Dataset</th>
<th>Control Dataset</th>
</tr>
</thead>
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<tr>
<td></td>
<td>MQ</td>
<td>AP</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>All Cases</td>
<td>933 (100)</td>
<td>450 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>489 (52.4)</td>
<td>198 (44.0)</td>
</tr>
<tr>
<td>Female</td>
<td>418 (44.8)</td>
<td>224 (49.8)</td>
</tr>
<tr>
<td>Unknown</td>
<td>26 (2.8)</td>
<td>28 (6.2)</td>
</tr>
<tr>
<td>Age Group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;18</td>
<td>51 (5.5)</td>
<td>35 (7.8)</td>
</tr>
<tr>
<td>18-65</td>
<td>595 (63.8)</td>
<td>225 (50)</td>
</tr>
<tr>
<td>65+</td>
<td>287 (30.8)</td>
<td>190 (42.2)</td>
</tr>
</tbody>
</table>

Note: Across all strata, proportions varied significantly by drug, in both the primary dataset and in the pooled data overall (p<0.05 by $\chi^2$ test).

Table 3.2. Drug Indication of Study Cases in the Primary Dataset, By Drug

<table>
<thead>
<tr>
<th></th>
<th>Primary Dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MQ</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>All Cases</td>
<td>933 (100)</td>
</tr>
<tr>
<td>Drug Indication</td>
<td></td>
</tr>
<tr>
<td>Prophylaxis</td>
<td>294 (31.5)</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>639 (65.5)</td>
</tr>
</tbody>
</table>

Note: Proportions varied significantly by drug, (p<0.001 by $\chi^2$ test).
Table 3.3. Prevalence of Reported Reactions by MedDRA SOC, by Drug and Overall, Primary Dataset

<table>
<thead>
<tr>
<th>SOC</th>
<th>MQ</th>
<th>AP</th>
<th>DX</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(%)</td>
<td>n</td>
<td>(%)</td>
</tr>
<tr>
<td>Any SOC</td>
<td>933</td>
<td>(100)</td>
<td>450</td>
<td>(100)</td>
</tr>
<tr>
<td>Blood and lymphatic system disorders</td>
<td>55</td>
<td>(5.6)</td>
<td>42</td>
<td>(9.3)</td>
</tr>
<tr>
<td>Cardiac disorders</td>
<td>136</td>
<td>(14.6)</td>
<td>19</td>
<td>(4.2)</td>
</tr>
<tr>
<td>Ear and labyrinth disorders*</td>
<td>33</td>
<td>(3.5)</td>
<td>6</td>
<td>(1.3)</td>
</tr>
<tr>
<td>Endocrine disorders*</td>
<td>7</td>
<td>(0.8)</td>
<td>2</td>
<td>(0.4)</td>
</tr>
<tr>
<td>Eye disorders</td>
<td>67</td>
<td>(7.2)</td>
<td>25</td>
<td>(5.6)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>233</td>
<td>(25.0)</td>
<td>123</td>
<td>(27.3)</td>
</tr>
<tr>
<td>Hepatobiliary disorders</td>
<td>36</td>
<td>(3.9)</td>
<td>26</td>
<td>(5.8)</td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>67</td>
<td>(7.2)</td>
<td>33</td>
<td>(7.3)</td>
</tr>
<tr>
<td>Musculoskeletal and connective tissue disorders</td>
<td>117</td>
<td>(12.5)</td>
<td>33</td>
<td>(7.3)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>438</td>
<td>(47.0)</td>
<td>131</td>
<td>(29.1)</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>527</td>
<td>(56.5)</td>
<td>95</td>
<td>(21.1)</td>
</tr>
<tr>
<td>Renal and urinary disorders</td>
<td>41</td>
<td>(4.4)</td>
<td>20</td>
<td>(4.4)</td>
</tr>
<tr>
<td>Respiratory, thoracic and mediastinal disorders</td>
<td>127</td>
<td>(13.6)</td>
<td>45</td>
<td>(10.0)</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>148</td>
<td>(15.9)</td>
<td>106</td>
<td>(23.6)</td>
</tr>
<tr>
<td>Vascular disorders</td>
<td>66</td>
<td>(7.1)</td>
<td>26</td>
<td>(5.8)</td>
</tr>
</tbody>
</table>

Note: Across all SOCs, proportions varied significantly by drug (p<0.05 by chi-square test). MedDRA—Medical Dictionary for Regulatory Activities, SOC—system organ class, MQ—mefloquine, AP—atovaquone-proguanil, DX—doxycycline, LCA—latent class analysis.

*SOC with 2.0% prevalence or less overall excluded from subsequent SOC-Level LCA.
Table 3.4. Prevalence of Reported Reactions by MedDRA Neuropsychiatric HLGT, by SOC-Level Model Most-Likely Neuropsychiatric Class, and Overall

<table>
<thead>
<tr>
<th></th>
<th>Partial</th>
<th></th>
<th>Partial</th>
<th></th>
<th></th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moderate</td>
<td>Mild</td>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Any neuropsychiatric HLGT</td>
<td>511 (93.4)</td>
<td>465 (92.0)</td>
<td>1,577 (89.6)</td>
<td>1,878 (43.0)</td>
<td>2,429 (45.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CNS infections</td>
<td>11 (2.2)</td>
<td>18 (1.0)</td>
<td>23 (0.5)</td>
<td>39 (0.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CNS vascular disorders</td>
<td>29 (5.7)</td>
<td>68 (3.9)</td>
<td>81 (1.9)</td>
<td>134 (2.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Communication disorders and disturbances**</td>
<td>31 (6.1)</td>
<td>75 (4.3)</td>
<td>73 (1.7)</td>
<td>126 (2.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Congenital neurological disorders</td>
<td>3 (0.6)</td>
<td>3 (0.2)</td>
<td>3 (0.1)</td>
<td>8 (0.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deliria (including confusion)**</td>
<td>60 (11.7)</td>
<td>128* (7.3)</td>
<td>135* (3.1)</td>
<td>219 (4.1)</td>
<td></td>
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<tr>
<td></td>
<td>Dementia and amnestic conditions**</td>
<td>37 (7.2)</td>
<td>92* (5.2)</td>
<td>92* (2.1)</td>
<td>161 (3.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Demyelinating disorders</td>
<td>5 (1.0)</td>
<td>19 (1.1)</td>
<td>26 (0.6)</td>
<td>32 (0.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depressed mood disorders and disturbances**</td>
<td>89 (17.4)</td>
<td>184* (10.4)</td>
<td>192* (4.4)</td>
<td>340 (6.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Developmental disorders NEC</td>
<td>0 (0.0)</td>
<td>1 (0.1)</td>
<td>1 (0.0)</td>
<td>1 (0.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dissociative disorders</td>
<td>6 (1.2)</td>
<td>16* (0.9)</td>
<td>16* (0.4)</td>
<td>25 (0.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eating disorders and disturbances</td>
<td>29 (5.7)</td>
<td>31 (1.8)</td>
<td>36 (0.8)</td>
<td>73 (1.4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Encephalopathies</td>
<td>15 (2.9)</td>
<td>16 (0.9)</td>
<td>20 (0.5)</td>
<td>43 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Headaches</td>
<td>131* (25.6)</td>
<td>121* (26.0)</td>
<td>190 (10.8)</td>
<td>242* (5.6)</td>
<td>415 (7.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Impulse control disorders NEC</td>
<td>2 (0.4)</td>
<td>12 (0.7)</td>
<td>12 (0.3)</td>
<td>16 (0.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increased intracranial pressure</td>
<td>11 (2.2)</td>
<td>50 (2.8)</td>
<td>54 (1.2)</td>
<td>70 (1.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Manic and bipolar mood disorders</td>
<td>7 (1.4)</td>
<td>40* (2.3)</td>
<td>40* (0.9)</td>
<td>55 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Movement disorders (including parkinsonism)</td>
<td>61* (11.9)</td>
<td>127 (7.2)</td>
<td>141* (3.2)</td>
<td>230 (4.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nervous system neoplasms benign</td>
<td>0 (0.0)</td>
<td>2 (0.1)</td>
<td>2 (0.0)</td>
<td>4 (0.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nervous system neoplasms malignant</td>
<td>0 (0.0)</td>
<td>2 (0.1)</td>
<td>3 (0.1)</td>
<td>6 (0.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neurological disorders of the eye</td>
<td>47* (9.2)</td>
<td>112 (6.4)</td>
<td>130* (3.0)</td>
<td>204 (3.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Neuromuscular disorders**</td>
<td>43 (8.4)</td>
<td>67 (3.8)</td>
<td>82* (1.9)</td>
<td>163 (3.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Peripheral neuropathies**</td>
<td>30 (5.9)</td>
<td>32 (1.8)</td>
<td>44 (1.0)</td>
<td>116 (2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psychiatric and behavioural symptoms</td>
<td>26* (5.1)</td>
<td>77* (4.4)</td>
<td>76* (1.7)</td>
<td>119 (2.2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psychiatric disorders NEC**</td>
<td>47 (9.2)</td>
<td>71 (4.0)</td>
<td>74* (1.7)</td>
<td>148 (2.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seizures (including subtypes)**</td>
<td>40 (7.8)</td>
<td>136 (7.7)</td>
<td>151 (3.5)</td>
<td>203 (3.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sexual dysfunctions and disturbances</td>
<td>13 (2.5)</td>
<td>18 (1.0)</td>
<td>19 (0.4)</td>
<td>46 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Sleep disturbances (including subtypes)***</td>
<td>0 (0.0)</td>
<td>1 (0.1)</td>
<td>1 (0.0)</td>
<td>1 (0.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Somatoform and factitious disorders</td>
<td>20 (3.9)</td>
<td>16 (0.9)</td>
<td>15 (0.3)</td>
<td>48 (0.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spiral cord and nerve root disorders</td>
<td>16 (3.1)</td>
<td>7 (0.4)</td>
<td>10 (0.2)</td>
<td>57 (1.1)</td>
<td></td>
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<tr>
<td>Structural brain disorders</td>
<td>8 (1.6)</td>
<td>10 (0.6)</td>
<td>12 (0.3)</td>
<td>34 (0.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Excludes 13 neuropsychiatric HLGTs with evidence of significant differential measurement by drug in each model by class. MedDRA—Medical Dictionary for Regulatory Activities, HLGT—high level group term, SOC—system organ class, CNS—central nervous system, NEC—not elsewhere classified

* HLGT-level indicators with evidence of significant differential measurement by drug in model class
** HLGT-level indicator selected for the final model
***Includes only those reactions not primarily categorized in the psychiatric disorders SOC
Table 3.5. Conditional Probabilities by Class, 2-Class HLGT-Level Model

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Non-Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication disorders and disturbances</td>
<td>0.182</td>
</tr>
<tr>
<td>Deliria (including confusion)</td>
<td>0.827</td>
</tr>
<tr>
<td>Dementia and amnestic conditions</td>
<td>0.186</td>
</tr>
<tr>
<td>Depressed mood disorders and disturbances</td>
<td>0.318</td>
</tr>
<tr>
<td>Neuromuscular disorders</td>
<td>0.090</td>
</tr>
<tr>
<td>Peripheral neuropathies</td>
<td>0.082</td>
</tr>
<tr>
<td>Psychiatric disorders NEC</td>
<td>0.314</td>
</tr>
<tr>
<td>Seizures (including subtypes)</td>
<td>0.181</td>
</tr>
</tbody>
</table>

Note: HLGT — high level group term, NEC – not elsewhere classified

Table 3.6: Prevalence of Most Commonly Reported HLGTs and Reactions within Each by PT, Among Cases Assigned to Most-Likely Syndrome Class, 2-Class HLGT-Level LCA Model, by Class

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Syndrome</th>
<th>Non-Syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(%)</td>
</tr>
<tr>
<td>All Cases</td>
<td>237</td>
<td>(100)</td>
</tr>
<tr>
<td>Deliria (including confusion)</td>
<td>181</td>
<td>(76.4)</td>
</tr>
<tr>
<td>Confusional state</td>
<td>114</td>
<td>(48.1)</td>
</tr>
<tr>
<td>Disorientation</td>
<td>55</td>
<td>(23.2)</td>
</tr>
<tr>
<td>Delirium</td>
<td>25</td>
<td>(10.5)</td>
</tr>
<tr>
<td>Neurological disorders NEC</td>
<td>109</td>
<td>(46.0)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>41</td>
<td>(17.3)</td>
</tr>
<tr>
<td>Hypoaesthesia</td>
<td>17</td>
<td>(7.2)</td>
</tr>
<tr>
<td>Paraesthesia</td>
<td>16</td>
<td>(6.8)</td>
</tr>
<tr>
<td>Vertigo</td>
<td>15</td>
<td>(6.3)</td>
</tr>
<tr>
<td>Anxiety disorders and symptoms</td>
<td>98</td>
<td>(41.4)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>55</td>
<td>(23.2)</td>
</tr>
<tr>
<td>Agitation</td>
<td>34</td>
<td>(14.3)</td>
</tr>
<tr>
<td>Panic attack</td>
<td>26</td>
<td>(11.0)</td>
</tr>
<tr>
<td>Sleep disorders and disturbances</td>
<td>84</td>
<td>(35.4)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>39</td>
<td>(16.5)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>21</td>
<td>(8.9)</td>
</tr>
<tr>
<td>Abnormal dreams</td>
<td>19</td>
<td>(8.0)</td>
</tr>
<tr>
<td>Nightmare</td>
<td>18</td>
<td>(7.6)</td>
</tr>
<tr>
<td>Sleep disorder</td>
<td>16</td>
<td>(6.8)</td>
</tr>
<tr>
<td>Depressed mood disorders and disturbances</td>
<td>74</td>
<td>(31.2)</td>
</tr>
<tr>
<td>Depression</td>
<td>62</td>
<td>(26.2)</td>
</tr>
</tbody>
</table>

Note: HLGT — high level group term; LCA—latent class analysis; NEC – not elsewhere classified; PT — preferred term
Figure 3.1. Study Cases in the Primary and Control Datasets

Note: MQ—mefloquine; AP—atovaquone-proguanil; DX—doxycycline; CQ—chloroquine; LP—loperamide.
Figure 3.2: Conditional Probabilities, Partially Conditionally Dependent (Partial) SOC-Level Model

Figure 3.3: Conditional Probabilities, Fully Conditionally Dependent (Full) SOC-Level Model

Note: blood—blood and lymphatic system disorders; cardiac—cardiac disorders; eye—eye disorders; gastro—gastrointestinal disorders; hep—hepatobiliary disorders; metab—metabolism and nutrition disorders; ms—musculoskeletal and connective tissue disorders; neuro—nervous system disorders; psych—psychiatric disorders; renal—renal and urinary disorders; resp—respiratory, thoracic and mediastinal disorders; derm—skin and subcutaneous tissue disorders; vascular—vascular disorders. Line thickness proportional to class membership probability.
CHAPTER 4
ASSOCIATION OF COMMON POLYMORPHISMS IN ABCB1 WITH
NEUROPSYCHIATRIC OUTCOMES IN U.S. MILITARY PERSONNEL
PRESCRIBED MEFLOQUINE: RESULTS OF A CASE-CONTROL
PHARMACOGENETIC STUDY

Abstract

The transmembrane drug transporter P-glycoprotein (P-gp), coded for by the ABCB1 gene, influences the partitioning of numerous psychoactive drugs across the blood-brain barrier. The lipophilic antimalarial drug mefloquine (MQ) exerts dose-dependent effects on multiple targets in the central nervous system (CNS) and is a well-characterized P-gp substrate and inhibitor. This study sought to evaluate whether common polymorphisms in ABCB1, which may predict altered P-gp mediated CNS drug efflux, were associated with certain surrogate phenotypes consistent with neuropsychiatric adverse reactions to MQ. A nested case-control study was performed among a population of 88,219 active duty U.S. military personnel prescribed MQ between 2001 and 2008, as identified from data in the Defense Medical Surveillance System (DMSS). 1,000 cases with various neuropsychiatric outcomes, and 1,930 controls without the outcome of interest, incidence-density matched on birth year, sex, and race were selected among a subset of 32,777 eligible subjects, restricted based on criteria designed to increase electronic visibility of potentially confounding mental health contraindications. Archived serum stored in the DoD Serum Repository (DoDSR) was used as a source of genetic material. Genotyping was performed by a commercial
laboratory. DNA yields of 0 µg to 46.75 µg (mean 0.107 +/- SE 0.916 µg) were obtained among the 2,930 0.5 ml specimens. Genotyping of three single nucleotide polymorphisms (SNPs) in ABCB1 – rs1128503 (C1236T), rs2032582 (G2677T/A), and rs1045642 (C3435T) – resulted in calls in 91.7%, 93.0%, and 93.5% of the samples, respectively. Significant associations were identified across a composite of all combined phenotypes with rs2032582, which is a non-synonymous SNP (p=0.048), and between a PTSD phenotype and the rs1128503/rs2032582/rs104564 TTC haplotype (p=0.027), although these associations were not replicated in more conservative genotyping. Polymorphisms in ABCB1 may be associated with MQ neuropsychiatric outcomes, but studies with more specific indicator diagnoses known to be positively associated with MQ use are needed to definitively address this hypothesis. This study demonstrates the potential utility of archived U.S. military electronic health data and serum for candidate pharmacogenetic studies, particularly for conditions with military relevance.

4.1. Introduction

Limited evidence supports a potential association between polymorphisms in the gene ABCB1 —which codes for the transmembrane drug transporter enzyme P-glycoprotein (P-gp) — and susceptibility to neuropsychiatric adverse reactions from the lipophilic antimalarial drug mefloquine (MQ), a well-characterized P-gp substrate and inhibitor that exerts dose-dependent effects on multiple targets in the central nervous system (CNS) (Aarnoudse et al., 2006; Nevin, 2011). Hypothesized neuropharmacokinetic mechanisms for such susceptibility involve either a dysfunction in baseline blood-brain barrier (BBB) P-gp mediated efflux of MQ, or a dysfunction in the
upregulation of P-gp expression in the BBB in response to increasing central nervous system (CNS) MQ concentrations (Nevin, 2012b, 2012c). Under these hypothesized mechanisms, silent and non-silent polymorphisms in the well-characterized coding region of P-gp, including common SNPs that may directly confer loss of P-gp-mediated BBB MQ efflux capacity, or that may be in strong linkage disequilibrium with polymorphisms in the promoter region and that result in loss of upregulation of BBB P-gp expression in response to MQ, could result in inadequate MQ efflux from the CNS, resulting in the drug reaching concentrations that cause significant adverse pharmacodynamic effects during prolonged prophylactic dosing.

Neuropsychiatric adverse reactions to MQ, when they occur, may not be initially recognized as being due to the drug, and may result in psychiatric care and the assignment of a range of psychiatric diagnoses (Nevin & Ritchie, 2015). Additionally, within the U.S. military, in deployed settings the sudden onset of such psychiatric symptoms, particularly those associated with suicidal ideation or psychosis, may prompt medical evacuation from deployment. Therefore, the identification of certain neuropsychiatric outcomes, such as suicide or medical evacuation, or the diagnosis of certain potential surrogate conditions, such as psychosis or posttraumatic stress disorder (PTSD), might be employed to identify U.S. military personnel potentially suffering from MQ adverse reactions.

Although the use of such surrogates would be expected to have low specificity for the syndrome of neuropsychiatric adverse reactions due to the drug, they might still be useful in association studies if the risk of the surrogate is increased with MQ use. For example, with the PTSD phenotype serving as an imperfect surrogate (Nevin & Ritchie,
2015; Nevin, 2015a) — even in deployed settings where MQ might be used, PTSD occurring independently of any effect of MQ would be expected to comprise the majority of incident cases — there may be sufficient power to detect genotypes or haplotypes that strongly increase the risk of MQ adverse reactions, should such reactions mimic and occasionally result in a diagnosis of PTSD.

This study therefore sought to evaluate whether common polymorphisms in ABCB1 were associated with diagnosis of PTSD and as well as certain other potential surrogate neuropsychiatric outcomes, including suicide, medical evacuation for mental health disorders, and diagnosis of psychosis, in a U.S. military population prescribed MQ.

4.2. Methods

A nested age-, sex,- and race-matched case-control pharmacogenetic study was conducted among U.S. military personnel using existing data stored in the Defense Medical Surveillance System (DMSS) and its related databases, and using commercial services to genotype routinely collected and existing linked serum stored in the Department of Defense Serum Repository (DoDSR) (Rubertone & Brundage, 2002). Both the DMSS and the DoDSR are operated by the Armed Forces Health Surveillance Branch (AFHSB) of the U.S. Defence Health Agency (DHA), and serum from the DoDSR, identified on the basis of medical and personnel data in the DMSS, may be utilized by civilian military-affiliated researchers for military-relevant research (Perdue, Cost, Rubertone, Lindler, & Ludwig, 2015; Perdue, Eick-Cost, & Rubertone, 2015). Well over 58 million specimens are currently housed in the DoDSR, collected
from over 10 million distinct individuals, most of whom were U.S. military service members at the time of collection (Russell, 2015). Owing to such repeated collection, and the entry of new individuals to the U.S. military, the DoDSR inventory grows at a rate of over 2 million specimens a year (Perdue, Cost, et al., 2015; Perdue, Eick-Cost, et al., 2015). Serum specimens contained in the DoDSR consist mostly of residual serum remaining from routine HIV serosurveillance. Although these specimens are stored in the DoDSR at -30°C after receipt and undergo multiple cycles of refrigeration, freezing, and thawing prior to their retrieval for research studies (Perdue, Eick-Cost, et al., 2015), based on the results of one prior study, they are a reliable source of amplifiable DNA for candidate gene studies (Scher et al., 2011).

The potential study population consisted of the entire cohort of U.S. military service members on active duty status at any time during the period 2001-2008, with one or more serum specimens available in the DoDSR. As the study investigator did not have direct access to the underlying data in DMSS and its related databases, nor to the stored serum in the DoDSR, research support staff at the AFHSB were provided with a detailed study protocol for implementing inclusion and exclusion criteria, case and control identification, creation of analytic datasets, and serum specimen selection, as summarized below.

4.2.1. Inclusion Criteria

Among the potential study population, inclusion in the study required one or more documented prescriptions of MQ within the military’s Pharmacy Data Transaction Service (PDTS) database, consisting of a minimum of 6 tablets of 250 mg MQ, generic or trade formulation, consistent with chemoprophylaxis, dispensed during the period 2001-
2008. The PDTS was recently integrated into DMSS and contains data on prescriptions dispensed at fixed medical facility pharmacies (Hurt & Zhong, 2015).

The prescription of MQ to those with certain prior neuropsychiatric conditions is contraindicated (Nevin et al., 2008), although prescribing of MQ to those with such contraindications has previously been reported in the U.S. military (Nevin, 2010). As prior neuropsychiatric conditions may worsen the confounding of any potential genetic association, to permit time for any such potentially contraindicating prior condition to have been electronically documented, inclusion in the study also required 12 months or greater of continuous, non-deployed active duty component time prior to the first prescription of MQ. To further improve electronic visibility of potentially contraindicating neuropsychiatric conditions, as evidenced by pharmaceutical usage or medical diagnosis in data contained in PDTS and DMSS, inclusion in the study cohort also required evidence of one or more additional prescriptions, not including MQ, in the 6 months prior to first receipt of MQ, and evidence of one or more medical encounters (including non-diagnostic administrative encounters) within each of the two sequential 6 month periods in the 12 months prior to first receipt of MQ, as determined by the presence of International Classification of Disease, 9th Edition, Clinical Modification (ICD-9-CM) diagnostic codes in any medical outcome data source in the DMSS. These stringent steps were performed to reduce potential bias from the inclusion of subjects receiving no electronic documentation of care, such as among personnel receiving care exclusively at aid stations, troop clinics, or onboard ships.
4.2.2. Exclusion Criteria

To avoid the confounding effects of potential neuropsychiatric adverse reactions due to malaria or due to treatment of malaria, individuals with evidence of a diagnosis of malaria (any ICD9-CM code of 084, in any medical outcome database, at any time) were excluded.

4.2.3. Case and Control Identification

Of the potential study subjects who met inclusion and exclusion criteria (i.e., eligible subjects), cases and controls for study were selected based on the following criteria. First, from all eligible subjects, up to 200 cases of suicide (denoted as “V” cases) were identified with a date of death occurring at any time within 2 years following the date of MQ prescription. Suicides were determined from the DMSS mortality database, which captures manner of death as provided to AFHSB from the Office of the Armed Forces Medical Examiner (OAFME). Second, up to 400-V cases with a mental illness (ICD-9CM codes 290-319) related medical evacuation (denoted as “W” cases) were identified with a date of evacuation occurring at any time within 2 years following the date of MQ prescription. Medical evacuations were determined by inclusion within the TRAC2ES medical evacuation database maintained by U.S. Transportation Command (USTRANSCOM). Third, up to 600-V-W cases of psychosis (denoted as “X” cases) were selected with an ICD-9CM diagnosis code of 295, 297, or 298 occurring within 2 years following the date of MQ prescription in any medical diagnosis data source available to AFHSB. Fourth, up to 800-V-W-X cases of PTSD (denoted as “Y” cases) were selected with and ICD-9CM diagnosis code of 309.81 occurring within 2 years following the date of MQ prescription in any medical diagnosis data source available to
AFHSB. Finally, up to 1000-V-W-X-Y total cases were selected from related psychiatric outcomes (denoted as “Z” cases) with the following ICD-9CM diagnosis codes occurring within 2 years following the date of MQ prescription in any medical diagnosis source available at AFHSB. These diagnosis codes corresponded to those from a previous analysis (Wells et al., 2006) and included 300.0, 300.2–300.3, and 300.89–300.9 for anxiety disorders; 296.00, 296.2–296.3, 296.40–296.99, 298.0, 300.4–300.5, and 311 for mood disorders; 308–309.4, and 309.82–309.9 for adjustment disorders; and 301 for personality disorders.

Within each of the categories of cases (V, W, X, Y, and Z), priority for selection was based on the following order: a single inpatient diagnosis code, two or more identical outpatient codes within 30 days (with the date of onset taken as the first date), and a single code. Priority was also given to select the maximum number of female cases, up to half of the specific number for that category, and then the remaining male cases were selected so as to produce the maximum number of suitable controls. When multiple diagnostic codes existed across equivalent patient encounters (e.g. inpatient encounters) the first code meeting criteria within the category was selected.

For all cases, at least one, and up to two controls, matched to cases on age (date of birth within one year), sex, and race (defined as white, black, or other), were selected from those who remained in active component status and lacked the specific outcome of interest for a period of time from the date of MQ prescription equivalent to the time between the date of prescription of MQ to the matched case and the date of the case event (herein described as the “index date”). Where sufficient continuous active component follow-up time did not exist, that potential subject was excluded as a control for that
particular case. In accordance with AFHSB protocols intended to simplify serum specimen retrieval, cases did not serve as controls for other cases.

4.2.4. Creation of Analytic Datasets

After implementing the study protocol inclusion and exclusion criteria and identifying cases and controls, the research support staff at AFHSB directed the retrieval of the designated serum specimens from the DoDSR and, upon confirming the availability of each specimen and the production or retrieval of aliquots, produced seven analytic datasets for the study investigator.

The first dataset linked anonymous subject identifier to anonymous specimen identifier row-wise in the format \{subject id, specimen id, specimen year\}. The subject id denoted subjects according to their category of outcome: suicides (labeled as “V” subjects), medical evacuations (“W” subjects), cases of psychosis (“X” subjects), cases of PTSD (“Y” subjects), and all other cases (“Z” subjects). Each subject id consisted of this alphanumeric prefix to which a unique three-digit case number was appended (e.g. “V001”). The subject id was further appended with a case-control flag identifying the subject as a case (“C”) or the first (“A”) or second (“B”) control. The specimen id was a unique 9 digit random number prefixed by the letter “S”, assigned to serve as the identifier for the corresponding DoDSR serum specimen. The specimen year corresponded to the year the specimen was collected from the subject and was to be used to assess possible systematic sources of variation in genotyping call rates.

The second dataset comprised a packing list linking specimen id to shipping information in the format \{rack, row, column\} corresponding to the position of each specimen in the shipping containers used by the DoDSR to ship the retrieved aliquots to
the commercial laboratory. This was to be used as necessary to assess additional possible systematic sources of variation in genotyping call rates.

The third dataset comprised basic subject demographic data, row-wise in the format \{subject id, sex, age, race\} where sex took on values “M” for male, “F” for female; age took on integer values representing case or control age as of the index date; and race took on values of “W” for white, “B” for black, and “O” for all other. Race was defined using self-reported data from DMDC based on traditional AFHSB business practices whereby any self-report of Hispanic ethnicity resulted in a Hispanic race value, leading to inclusion of the subject in the “O” category.

A fourth dataset provided a summary of case-defining event data row-wise in the format \{subject id, case latency, case type, index year, dx X\} where case latency took on integer values representing the number of days from case date of MQ prescription to index date; and case type took on values of “S”, “E”, “I”, “O2” and “O1”, where “S” denoted a suicide event, “E” a medical evacuation event, “I” a single inpatient diagnosis code, “O2” two or more identical outpatient codes within 30 days (with the date of onset taken as the first date), and “O1” a single code. The index year represented the year the case-defining event occurred. For all case-defining events except suicide, for which it was blank, dx (with a suffix X from 1 up to 8) represented the specific ICD-9CM diagnostic codes associated with the case-defining event.

A fifth dataset provided information necessary to address the potential for confounding due to prior contraindications or due to deployment. In this dataset, event dates were defined in terms of date of the subject MQ prescription. This dataset was row-wise in the format \{subject id, contraindication date, deployment form date, operation,
operation date. To populate the Contraindication Date field, records of medical
diagnosis data in DMSS and pharmaceutical usage from PDTS were reviewed according
to previously published methods for evidence of medical or pharmaceutical
contraindications to MQ use (Nevin, 2010), to include records of ICD-9CM codes in
DMSS within the 12 months prior to MQ prescription, or prescriptions in PDTS within
the 6 months prior to MQ prescription. If present, the date of the most recent
contraindicating event, prior to or including the date of MQ prescription, and relative to
the date of MQ prescription, was defined as the contraindication date. This was an
integer value and was always between -365 and zero. To populate the Deployment Form
Date, electronic records of Pre-deployment Health Assessments (Form DD2795) in
DMSS were also reviewed (Nevin, 2009), and the date of the most recent form, if present
anytime within the 12 months prior to or including the MQ prescription date, was defined
as the deployment form date, in days relative to the MQ prescription date. This was an
integer value and was always between -365 and zero. Lastly, records of deployments, as
identified in the Defense Manpower Data Center (DMDC) Contingency Tracking System
(CTS) roster, were also reviewed. If the records indicated one or more active
deployments at anytime during the period from the date of MQ prescription through the
date of MQ prescription plus the case latency period, the earliest such deployment would
be characterized in operation as either Operation Enduring Freedom (“OEF”), Operation
Iraqi Freedom (“OIF”), or other, and blank otherwise. The start date of the earliest such
deployment was characterized in operation date in days relative to the MQ prescription
date. Operation date was to be an integer and was always to be less than or equal to the
case latency. As electronically documented MQ prescriptions almost always occur prior to deployment, the operation date would also be expected to be mostly positive.

A sixth dataset provided information necessary for better compliance with the requirements of the STROBE and RECORD checklists (Benchimol et al., 2015; Vandebroucke et al., 2007). The dataset consisted of counts of potential subjects at various stages of application of the inclusion and exclusion criteria and case and control selection, further divided by sex using the suffix m and f at each variable. The number potentially eligible (N01) was the number of U.S. military service members with any period of active duty status during the period 2001 to 2008 and with serum available within the DoDSR. The number prescribed MQ (N02) was, of N01, the number with one or more qualifying prescriptions for MQ. The number of active duty, non-deployed prescribed MQ (N03) was, of N02, the number with 12 months or greater of continuous, non-deployed active duty time prior to this prescription. The number with diagnosis visibility (N04) was, of N03, the number with some medical diagnosis in each of the two 6 month periods in the 12 months prior to receipt of MQ. The number not excluded due to malaria (N05) was, of N04, the number not excluded for any evidence of diagnosis of malaria. Of these, the number with pharmacy visibility (N06) was, of N05, the number with some other prescription in the 6 months prior to MQ receipt. This number, N06, was considered the number of potential study subjects eligible for selection as cases or controls. Of these, the number of potential V cases (NVX) was the total number of potential cases meeting criteria for V case inclusion (without regard to potential selection as a case in another category), while number of potential V controls (NVC) was the number of potential controls when matched. Similar figures were also provided for W, X,
Y, and Z cases using variable names \textit{NWX, NWC, NXX, NXC, NYX, NYC, NZC and NZX}, as appropriate.

A final seventh dataset consisted of a demographic breakdown of the counts of \textit{N01f/m, N02f/m, N03f/m, N04f/m, N05f/m, and N06f/m} by race.

4.2.5. Serum Specimen Selection

For each study subject, research support staff at AFHSB identified the most recently collected serum specimen available within the DoDSR inventory. Original parent specimens or, where available, previously produced 0.5mL aliquots were manually retrieved from frozen storage by staff at the DoDSR. Parent specimens from which aliquots were not previously produced were thawed and manually agitated, and a 0.5mL aliquot was produced using automated liquid handling devices, and subsequently refrozen for shipment. Upon their retrieval for production, all study specimens were labeled only with the anonymous specimen ID assigned by AFHSB research support staff.

4.2.6. DNA Extraction and SNP Genotyping

DNA extraction and SNP genotyping were performed at the commercial laboratory (Bioserve, Beltsville, MD), according to previously described methods (Scher et al., 2011), as provided by the laboratory.

Briefly, DNA extraction from the thawed frozen serum samples was performed using Machery-Nagel, Inc. Nucleospin Blood L kit (Machery-Nagel, Germany) per the manufacturer’s specifications. Quantification of DNA mass was performed using reverse transcriptase polymerase chain reaction (RT-PCR) with primers for the human-specific RNaseP gene with 6-FAM at the 5’ end, and a Tamara quencher at the 3’ end of the
probe. SNP genotyping was performed using the MassARRAY iPLEX platform, a PCR process and mass spectrometry-based system (Sequenom, CA).

The first step in the SNP genotyping process was to perform PCR to amplify the region surrounding each SNP. PCR reactions were performed in a total volume of 5 µl using 0.625 µl of 10X PCR buffer (Solis Biodyne, Estonia), 1.0 µl of forward and reverse primer mix of all grouped SNP assays (0.5 pm/µl of each primer), 0.325 µl MgCl₂ (25 mM), 0.1 µl deoxynucleotide triphosphates (dNTPs) (2.5 mM each), 0.1 µl of Taq polymerase enzyme (Solis Biodyne), and 2.85 µl of sterile water. The amplification program consisted of 95°C for 15 minutes (activation of Taq enzyme), 45 cycles of 95°C for 20 second (denaturation), 56°C for 30 seconds (annealing) and 72°C for 1 minute (extension), and a final extension temperature of 72°C for 3 min before cooling at 4°C.

The second step was to treat the PCR reactions with shrimp alkaline phosphatase (SAP) to dephosphorylate unincorporated nucleotides, and prevent their future incorporation and interference with the primer extension step. The heat labile SAP was then heat inactivated. The reaction used 0.17 µl of hME buffer, 0.3 µl of SAP enzyme (Sequenom) and sterile, nanopure water to a volume of 2 µl. When added to the PCR product, the final reaction volume was 7 µl. The thermal cycler program consisted of three steps: a hold at 37°C for 20 minutes, a hold at 85°C for 5 minutes, and a final cooling step of 4°C.

The third step was to perform a base extension reaction using thermo sequenase to incorporate mass modified dideoxy nucleotides. This created a mass difference that would lead to a significantly different desorption-ionization during mass spectrometry analysis. In general, the reaction required 0.804 µl of extension primer mix
(approximately 7-14 pM/µl of each primer), 0.2 µl of iPLEX termination mix, 0.041 µl of TERMIPol enzyme (Solis Biodyne) and sterile, nanopure water to a volume of 2 µl. The iPLEX cocktail mix was added to the cleaned PCR product for a final reaction volume of 9 µl. The extension thermal cycler conditions consisted of 94°C for a 30 second hold, followed by 40 cycles of 94°C for 5 seconds, 52°C for 5 seconds, and 80°C for 5 seconds. A final extension was performed at 72°C for 3 minutes and then cooled to 4°C.

All PCR and extension primers were synthesized at the commercial laboratory. The synthesized primers for PCR and extension reactions were first made to concentrations of 100pM/µl and 300pM/µl respectively. Each of the primers was aliquoted into a separate tube and the concentration adjusted to 2pM/µl. These primers were then spotted over a Sequenom chip and analyzed using a MALDI-TOF mass spectrometer. The results were checked using Sequenom’s OligoCHECK software. All the peaks and results were manually checked. If any of the primers failed QC they were synthesized again. After all the primers were deemed acceptable they were used for setting up the validation of the assays.

All the PCR primers were mixed together and diluted so that each primer was at a concentration of 0.5pM/µl. The extension primers were then mixed to form different concentrations according to their mass and concentrations, varying between 7pM/µl to 14pM/µl. After mixing, the extension primers were diluted and mixed with ion-exchange resin to clean any salts. After cleaning, the primer mix was spotted on a SpectroChip and analyzed using SpectroTYPER (Sequenom). The peaks of the individual extension primers were noted and their peak intensities were adjusted linearly by adjusting the
concentrations of each primer in the mix. The extension primer mix was optimized when all the peaks of the primers were satisfactorily in range.

To optimize the mass spectrometry analysis of the reaction products, SpectroCLEAN resin was used to remove salt ions. A mixture of approximately 6mg of resin and 24 µl of water was added to each reaction and mixed for 15-20 minutes. Approximately 15 nl of each reaction product was transferred, or “spotted” onto corresponding elements of a silicon microchip forming a crystalline matrix. After the chip was introduced into a vacuum, each element of the chip was sequentially irradiated with ultraviolet (UV) laser pulses, leading to ionization of the analyte molecules. Ions were then accelerated through a detection region at a velocity inversely proportional to the mass to charge ratio.

All plex group assays were initially validated using 24 DNA samples, in duplicate, obtained from the Coriell Polymorphism Discovery Panel. This resource comprises DNA samples from 450 unrelated individuals, male and female, designed to reflect the diversity in the human population. The commercial laboratory used a predefined nested subset of 24 samples as positive controls, called PD controls, to establish the validity of its assays, selected based upon their genotypes to cover all the possible genotypes available for the multiplex assay groups developed.

During mass spectroscopy, the resultant peaks were first analyzed by SeQuenom’s automated software SpectroTYPER. Peaks of the assays were checked for intensities and other characteristics. The clusters of the whole validation samples were also analyzed and verified. Genotypes obtained from the validation experiment were compared to available online allele frequencies for confirmation, and duplicate sample calls were compared.
After checking all the above, the multiplex groups of assays are deemed validated and ready for scale up to be done over study samples.

When testing the study samples, DNAs were plated in received order onto 384-well plates. Four PD positive controls for each assay and four negative no template controls were included per plate. Peaks for individual assays were checked manually even if the Sequenom’s SpectroTYPER software made automated calls over them. All of the study samples were genotyped in duplicate.

Results of genotyping were provided to the study investigator by the commercial laboratory in 3 datasets, one per genotyped SNP, row-wise in the general format 
\{specimen id, commercial lab id, genotype call (run 1), genotype call (run 2)\}. The commercial laboratory also provided an additional dataset providing information on DNA extraction yield, in the general format \{specimen id, commercial lab id, yield\}.

4.2.7. DNA Extraction and SNP Genotyping Pilot Analysis

To confirm the adequacy of the intended 0.5 ml serum volume for DNA extraction yield, and to confirm satisfactory call rate and concurrence and reliability of the SNP genotyping, a pilot analysis was performed prior to retrieval of the substantive study specimens.

A set of 10 “orphaned” specimens from the DoDSR inventory (unlinked to any demographic data) were retrieved, and each was divided into two aliquots (of 1.0 ml and 0.5 ml respectively) linked to the original specimen identifier. These aliquots were provided to the commercial laboratory blinded to linkage, and DNA yields and genotyping results for the 3 SNPs from each aliquot were provided by the commercial laboratory according to the specified laboratory methods.
4.2.8. Statistical Analysis

Normality of DNA yields in pilot analysis was assessed by Shapiro-Wilk test and distribution of DNA yields by volume was compared by Wilcoxon matched-pairs signed-ranks test. Pilot analysis of SNP genotyping reliability was assessed by kappa statistic.

Sex and race distributions of the original potential study population and the MQ-prescribed populations were compared with the study population of cases and controls by means of chi-square test.

Normality of DNA yields and log-transformed yields in the substantive study were assessed by Shapiro-Wilk test. DNA yields were compared across case and control status by Wilcoxon rank-sum test. The association of genotyping calls at one or more SNP with DNA yield was assessed by logistic regression.

Results of genotyping across all combined phenotypes were assessed by Hardy-Weinberg equilibrium tests, and all possible haplotypes were estimated according to published methods using PLINK 1.07 (Purcell et al., 2007). The most likely phased haplotypes were used in subsequent analyses.

Genetic association tests of matched cases and controls were performed for a composite of all combined phenotypes versus all controls and separately for each individual case phenotype versus the matched controls using conditional logistic regression with robust standard errors, reporting unadjusted p-values for tests of genotypes and haplotype association under an additive model. Owing to sparse data in certain individual phenotypes, the analyses were repeated on unmatched cases and controls using Firth’s penalized maximum likelihood estimation method.
Significant associations demonstrated in a prior study (Aarnoudse et al., 2006) were then examined more closely by testing associations of the PTSD phenotype with minor allele genotypes and haplotypes in a recessive model, stratified by sex. This phenotype was selected for analysis based on a large phenotypic sample size, with matched case control power calculations for this analysis performed approximating 2 matched controls per case. Unless otherwise specified, all statistical tests were conducted using *STATA Version 14* (StataCorp, College Station, TX).

### 4.2.9. Informed Consent and Subject Non-Identifiability

This study involved analysis of data and serum specimens that were previously collected for non-research, public health purposes. The study investigator was provided only with analytic datasets produced by staff at AFHSB identified by anonymous subject and sample ID, and the results of genotyping as provided by the commercial laboratory identified by sample ID. In accordance with 32 CFR 219.102(f) and 45 CFR 46.102, the study was deemed exempt from institutional review board review by the U.S. Army Medical Research Material Command (HRPO Log Number A-16715, 24 February 2011) and the Johns Hopkins Bloomberg School of Public Health (20 April 2012).

In accordance with standard protocols developed to ensure non-identifiability of subjects, research support staff at AFHSB securely deleted all linkages to the original identified data on receipt of the serum specimens by the commercial laboratory, at which time the analytic datasets were provided to the investigator. Serum specimens were destroyed by the commercial laboratory at the time the results of genotyping were provided to the study investigator.
4.3. Results

4.3.1. Pilot DNA Yield and SNP Genotyping Reliability

DNA yields for the orphan specimens ranged from 0.038 µg to 1.54 µg (mean 0.609 +/- SE 0.211 µg) in the ten 1 ml aliquots and from 0.0162 µg to 1.12 µg (mean 0.272 +/- SE 0.124 µg) in the ten 0.5 ml aliquots. These yields were non-normally distributed in each set of aliquots (p=0.001 and 0.002, respectively), and they were significantly different from each other (p=0.013) in matched analysis. There was a higher DNA yield from the 1.0 ml specimen than from the 0.5ml in 9 of 10 of the original matched specimens.

SNP genotyping, performed in duplicate, resulted in concordant calls for all 20 specimens at all 3 SNPs. Genotyping at rs1128503 and rs2032582 concurred for all 10 pairs of matched 0.5 ml and 1.0 ml specimens, but differed at rs1045642 for one pair of matched specimens (kappa=0.851). The 0.5 ml specimen (genotyped as CT) and the 1.0 ml specimen (genotyped as TT) had DNA yields at the 25th and 75th percentiles of their volumes, respectively.

These results were deemed acceptable to proceed and use 0.5 ml specimens for the study.

4.3.2. Study Sample

The potential study population consisted of 2,961,927 individuals. Of these, 88,219 (3.0%) had a qualifying prescription for MQ, and of these, 64,508 (73.1%) were prescribed the drug after a minimum of 12 months continuous active duty time. Of these, 46,020 (71.3%) had electronic documentation of a medical encounter in each of the two consecutive 6 month periods prior to the qualifying prescription. From these subjects, a
total of 304 individuals were excluded due to diagnosis of malaria. This resulted in
45,716 potential study subjects whose prescription records were subsequently reviewed
to identify those with one or more additional prescriptions, not including MQ, in the 6
months prior to first receipt of MQ. Application of this step resulted in 32,777 of these
(71.7%, or 1.1% of the original potential study population, and 37.2% of the MQ-
prescribed population) being retained as potential study subjects eligible for case and
control selection, as shown in Figure 4.1.

This eligible population differed significantly from the original potential study
population by sex (p<0.001) and race (p<0.001), and also from the MQ-prescribed
population by sex (p<0.001) and race (p<0.001), as shown in Table 4.1.

Application of inclusion and exclusion criteria resulted in fewer cases of suicide,
medical evacuation for mental disorders, and psychosis being identified than anticipated
during protocol design. Sequential selection of cases in subsequent phenotypes from
among previously unselected cases, and study limits on maximum case population,
resulted in fewer cases of all phenotypes (except suicide) being selected than were
available. However, well over double the number of cases of PTSD and other disorders
were identified than planned. Overall, 93% of cases were matched to paired controls and
the remainder were matched to singleton controls, as shown in Table 4.2. Differential
matching to singleton controls by sex and race resulted in non-statistically significant
differences in the overall sex and race composition by case and control status, as shown
in Table 4.3.
4.3.3. DNA Yields

DNA yields in the substantive study ranged from 0 µg to 46.75 µg (mean 0.107 +/- SE 0.916 µg) among the 2,930 0.5 ml specimens. A total of 30 specimens had no measurable DNA yield, 21 yielded between 1 and 4 µg, and 2 yielded over 10 µg. Both yields and log-transformed yields were non-normally distributed (p<0.001). DNA yields did not vary significantly by case or control status (p=0.298).

4.3.4. SNP Genotyping Results

Genotyping of rs1128503 resulted in bi-allelic (C/T) calls, with successful calls in 2,507 and 2,508 specimens (85.6%) in the first and second run, respectively. Of these, 181 and 182 were non-calls in the other run, respectively, and 2 specimens had discordant calls (kappa=0.822). In a liberal analysis, treating concordant calls and unduplicated calls as valid and concordant non-calls or discordant calls as non-calls (i.e., missing) resulted in calls for 2,687 specimens (91.7%). In a conservative analysis, treating only concordant calls as valid and everything else as non-calls (i.e., missing) resulted in calls for 2,324 specimens (79.3%).

Genotyping of rs2032582 resulted in tri-allelic (A/G/T) calls as expected, with successful calls in 2,542 (86.8%) and 2,554 (87.2%) specimens during the first and second runs, respectively. Of these, 183 and 195 were non-calls in the other run, respectively, and 13 specimens had discordant calls (kappa=0.813). There were 50 specimens with concordant AG calls and 41 specimens with concordant AT calls. During the first and second runs, there were 10 and 11 specimens, respectively, with AG calls and non-calls in the other run; and 3 and 2 specimens, respectively, with AT calls and non-calls in the other run. To facilitate bi-allelic haplotype imputation, the 103 and 106
with an A allele in the first and second run, respectively, were recategorized. Liberally
recategorizing each of these A alleles as a T, treating other concordant calls and
unduplicated calls as valid, and treating only concordant non-calls or discordant calls as
non-calls resulted in calls for 2,724 specimens (93.0%). Conservatively recategorizing
each of the A allele containing calls as a non-call, and treating non-calls in either run or
discordant calls as non-calls resulted in calls for 2,255 specimens (77.0%).

Genotyping of rs1045642 unexpectedly resulted intri-allelic (A/C/T) calls, with
successful calls in 2,532 (86.4%) and 2,515 (85.8%) specimens during the first and
second runs, respectively. Of these, 239 and 222 were non-calls in the other run,
respectively, and 14 specimens had discordant calls (kappa=0.767). There was a call
containing an A allele in one unique specimen in each run (one AA and one AT), while
the other run of each specimen resulted in a non-call. Liberally recategorizing each of
these A alleles as a T, treating concordant calls and unduplicated calls as valid, and
treating only concordant non-calls or discordant calls as non-calls resulted in calls for
2,740 specimens (93.5%). On conservatively re-categorizing each of the A allele
containing calls as a non-call, and treating non-calls in either run or discordant calls as
non-calls resulted in calls for 2,279 specimens (77.8%).

4.3.5. Genotyping Call Rates by Yield

Six of 30 specimens with no measurable DNA yield resulted in calls at one or
more SNPs in the conservative analysis, while 25 of 30 specimens with no measurable
DNA yield resulted in calls at one or more SNPs in the liberal analysis. DNA yield was
linearly associated with success of genotyping call at one or more SNP in the
conservative (p=0.012) and liberal (p=0.001) genotyping analyses.
4.3.6. Genotyping Results

Results of genotyping, reflecting the re-categorization of discordant and A allele calls, are presented for rs1128503 in Table 4.4, rs2032582 in Table 4.5, and rs1045642 in Table 4.6. The variants rs1128503 and rs1045642 were both in Hardy-Weinberg equilibrium among cases and controls in the liberal and conservative genotype analysis. In contrast, rs2032582, was notably out of equilibrium (p=0.002) among controls for the composite phenotype in the liberal genotype analysis, and among both cases (p=0.024) and controls (p=0.013) in the conservative genotype analysis.

4.3.7. Haplotype Estimation

In the liberal genotyping analysis, haplotypes were estimated for 2,748 (93.8%) of the 2,930 subjects. The most likely haplotypes were assigned with greater than 95% probability for 2,451 (89.2%) subjects. In contrast, in the conservative genotyping analysis, haplotypes were estimated for only 2,382 (81.3%) of the 2,930 subjects, and the most likely haplotype was assigned with greater than 95% probability among only 1,930 (81.0%) of these subjects.

4.3.8. Genotype/Haplotype Association Results

In the liberal genotyping analysis, there were significant associations of the composite case phenotype with rs2032582 (T vs G, OR=0.89, p=0.048) and haplotypes of rs1128503/rs2032582/rs1045642 (TTC vs all other haplotypes, OR=0.57, p=0.027). With regard to individual case phenotypes, there were nominally significant associations of the PTSD phenotype with the rs1128503/rs2032582/rs1045642 haplotype (TTC vs all other haplotypes, OR=0.38, p=0.021), and of the medical evacuation phenotype with the rs1128503/rs2032582/rs1045642 haplotype (CGC vs all other haplotypes, OR=1.89,
p=0.041). However, in the conservative genotyping analysis, none of the associations observed in the liberal genotyping analysis were observed, and only one new association was nominally significant: this was between the medical evacuation phenotype and rs1045642 (T vs C, OR=0.43, p=0.027). These results are shown in Tables 4.7 and 4.8.

Using Firth’s penalized logistic regression to account for sparse data with certain genotypes and haplotypes, the association tests that were significant in conditional logistic regression in both the liberal and conservative genotyping analyses remained significant. However, no additional genotype or haplotype associations were identified as significant with those phenotypes for which conditional logistic regression models could not be run, as shown in Tables 4.9 and 4.10.

4.3.9. Minor Allele Genotype and Haplotype Association with PTSD in a Recessive Model

In contrast to findings from a previous study (Aarnoudse et al., 2006), there were no significant associations between neuropsychiatric adverse effects as determined by the PTSD phenotype and homozygous rs1128503/rs2032582/rs1045642 TTT haplotype or homozygous TT genotypes at any of the individual variants either using the full sample or in strata by sex, as shown in Table 4.11.

4.3.10. Power Analysis

Given control prevalence for the rs1128503 TT, rs2032582 TT, and rs1045642 TT genotype of 16.5%, 17.2%, and 20.6% respectively, and for the homozygous rs1128503/rs2032582/rs1045642 TTT haplotype of 12.0%, and assuming 2 controls per case based on the total number of cases with valid genotypes or haplotypes in the liberal genotyping analysis, the study had 80% power to detect a significant odds ratio in the
PTSD phenotype analysis of 1.29 for the rs1128503 TT genotype, 1.28 for the rs2032582 TT genotype, 1.26 for the rs1045642 TT genotype, and 1.33 for the rs1128503/rs2032582/rs1045642 haplotype.

4.4. Discussion

This nested case-control pharmacogenetic study examined ABCB1 genotype and haplotype associations with various mental health outcomes consistent with neuropsychiatric adverse reactions to MQ among a U.S. military population prescribed the drug. It found nominal evidence of associations between certain phenotypes and polymorphisms in the gene that codes for the MQ BBB drug transport enzyme P-gp.

Despite these intriguing findings, these results must be interpreted with caution for at least three reasons. First, certain of the observed associations, including of the rs1128503/rs2032582/rs1045642 TTC haplotype with the PTSD phenotype and of rs2032582 with the composite case phenotype, were each inverse, suggesting a protective effect which is inconsistent with the previously hypothesized mechanism whereby specific polymorphisms would increase risk through altered P-gp mediated CNS drug efflux (Aarnoudse et al., 2006; Nevin, 2012b, 2012c). Such a protective effect would imply an otherwise common elevated risk of the various phenotypes, including PTSD diagnosis, with all but the specific genotype or haplotype — a seemingly unlikely outcome.

Second, none of the findings in liberal genotyping analysis, including the elevated risk of the medical evacuation phenotype observed with rs1128503/rs2032582/rs1045642 CGC haplotype, were consistent in an analysis using more conservative genotype data,
suggesting the possibility that these associations were spurious due to unreliable
genotyping and haplotype estimation. This concern is supported by findings of significant
deviation from Hardy-Weinberg equilibrium in rs2032582 among controls in the liberal
genotyping analysis.

Lastly, the findings of statistical significance did not control for multiple
hypothesis testing, increasing the likelihood of a Type I error. For example, there would
have been no significant genotype associations with the composite case phenotypes in the
liberal genotyping analysis at a Bonferroni-adjusted p-value of 0.017 after accounting for
the three polymorphisms evaluated ($\alpha=0.05/n$, $n=3$). Similarly, there would also have
been no significant haplotype associations with the composite case phenotypes in the
liberal genotyping analysis at a Bonferroni-adjusted p-value of 0.006 after accounting for
the eight possible haplotypes evaluated ($\alpha=0.05/n$, $n=8$). Although the eight haplotypes
were not strictly independent of the genotypes, and hence such a naïve Bonferroni
correction might be overly conservative, given that most significant unadjusted haplotype
association had a p-value of only 0.021, it is unlikely that this association would have
reached significance under another more sophisticated method of multiple testing
correction.

This study also did not find evidence to support the positive results of a prior MQ
pharmacogenetic study conducted in a non-military population (Aarnoudse et al., 2006),
which found evidence of associations between the homozygous
rs1128503/rs2032582/rs1045642 TTT haplotype, as well as homozygous TT genotypes at
each of the individual variants, and risk of broadly defined neuropsychiatric adverse
effects, among previously healthy female, but not male, subjects. In this earlier study,
neuropsychiatric adverse effects were identified on the basis of self-reported prodromal symptoms including abnormal dreams, insomnia, agitation or changes in mood (Aarnoudse et al., 2006).

In the current study, self-reported symptoms could not be directly assessed. Instead, it was postulated that owing to the low rate of discontinuation of MQ in military settings (Boudreau et al., 1993), prodromal symptoms would increase the risk of the more serious neuropsychiatric adverse reactions described in the MQ drug label (Nevin & Byrd, 2016), including suicide and psychosis, and of medical evacuation for these and other neuropsychiatric outcomes. Possibly owing to this study’s strict inclusion criteria, which were deemed desirable to increase the likelihood of identifying prior contraindications, this study identified fewer cases of suicide, medical evacuations, and psychosis than were anticipated, and this consequently resulted in more cases of other psychiatric disorders including PTSD being included as surrogate phenotypes.

In the absence of a specific diagnosis for the syndrome of neuropsychiatric adverse reactions due to the drug, this study therefore assumed that experiencing an adverse reaction to MQ would increase the risk of diagnosis of this condition (Nevin & Ritchie, 2015; Nevin, 2015a). While the increased risk, if any, of diagnosis of PTSD with MQ use is not known, such an increase is considered plausible. A single underpowered analysis, comparing deployed MQ users to non-MQ-using deployed cohorts, did find a non-significant increased hazard for hospitalization for PTSD of 1.66 (95% CI 0.21-12.85) (Wells et al., 2006). Although the current study was unable to independently calculate a similar relative risk—being limited to MQ users—there is evidence to suggest the risk of PTSD diagnosis among the study cohort may not have been
substantially higher than across the military as a whole. Among the 32,777 subjects in the eligible population with up to 2 years of follow-up, only 493 potential cases of PTSD were identified, equivalent to a minimum rate of 7.52 cases of PTSD per 1,000 per year. This is consistent with previously published rates of incident PTSD diagnosis (as opposed to positive symptom screening or diagnosis of other anxiety disorders) across the military as a whole, which rose monotonically from only 1.7 per 1,000 per year in 2001, to just 9.9 per 1,000 per year in 2009 (Armed Forces Health Surveillance Center, 2012). This calculation assumes that none of the subjects in the current study, all of whom were prescribed MQ, had a prior history of PTSD. Although 112 of the 444 of PTSD cases (25.2%) had evidence of one or more mental health contraindications prior to being prescribed the drug, the study’s methods did not identify the specific contraindicating condition, and hence cannot rule out a prior diagnosis of PTSD among this study’s assumed incident cases. For these reasons, although there is significant uncertainty, it cannot be ruled out that the study population may have had an even lower rate of incident PTSD diagnosis among MQ users than across the military as a whole.

Despite these limitations, even generously assuming the point estimate of 1.66 as the increased risk of any PTSD diagnosis with MQ use, at most, 39.8% of the PTSD cases in the current analysis would be considered attributable in any way to MQ use and hence plausibly associated with ABCB1 genotype or haplotype, with the remaining PTSD cases attributable to other exposures including deployment. Recalculating power in light of this smaller effective sample size, the current study had 80% power to detect an OR of 1.55 for PTSD diagnosis due homozygous rs1128503/rs2032582/rs1045642 TTT haplotypes. While this is still considerably less than the OR point estimate of 4.5 (95% CI
1.3—16.0) for broadly defined neuropsychiatric adverse effects associated with this haplotype observed in the earlier study (Aarnoudse et al., 2006), given the range of potential neuropsychiatric phenotypes associated with MQ use, the magnitude of any true association may have been too low for detection.

In retrospect, it appears that the use of such varied and relatively non-specific individual phenotypes in this study, including PTSD, may have resulted in insufficient power to detect any true association of neuropsychiatric adverse reactions with ABCB1 genotypes or haplotypes. Improvements in the characterization of diagnostic indicators for the MQ neuropsychiatric adverse reaction phenotype, such as through combinations of specific ICD-9-CM (or ICD-10) diagnosis codes for combinations of neurologic and psychiatric conditions not commonly seen in other disorders, may aid in improving power to observe such true associations in future retrospective studies.

Despite its negative findings, this is the first study to successfully employ stored DoDSR serum as a source of DNA for genotyping in which U.S. military subjects were identified retrospectively on the basis of past prescription of a particular drug. This pharmacogenetics study has therefore demonstrated the feasibility of using the data resources of the AFHSB to retrospectively identify U.S. military subjects with specific case phenotypes and with plausible prior drug exposure, and has further demonstrated the feasibility of obtaining sufficient quantities of DNA from serum stored in the DoDSR to amplify and support limited genotyping of one or more candidate genes (Scher et al., 2011). This study has also further demonstrated the feasibility of using commercial services to reliably perform DNA amplification and genotyping (Scher et al., 2011), and has provided estimates of potential case counts, DNA and genotyping yield, and
genotyping reliability that may be useful in planning for future candidate gene studies using DMSS data and DoDSR specimens.

Pharmacogenetic candidate gene studies that employ DoDSR specimens have certain advantages over other study designs, particularly for the investigation of conditions of U.S. military relevance. As the data and specimens provided to investigators are pre-existing and fully anonymous, studies may be performed as IRB-exempt non-human subjects research, significantly reducing the time from study conception to data acquisition. Such studies are also relatively affordable, costing the civilian investigator only the cost of specimens from the DoDSR (approximately US$20 per shipped specimen, inclusive of all costs of analysis by AFHSB, in 2010) (Moore et al., 2010), plus the costs of DNA amplification and genotyping.

While genome-wide association studies would be ideal for generating additional hypotheses and identifying potential additional candidate genes that may be associated with neuropsychiatric adverse reactions to MQ — particularly those genes which may plausibly mediate the drug’s neuropharmacokinetics — it is unclear whether serum from the DoDSR would be able to support whole genome amplification, and no published studies have yet assessed the feasibility of this technique using DoDSR specimens (Perdue, Cost, et al., 2015). Additionally, although studies involving DoDSR specimens are notionally anonymous, genome-wide amplification and genotyping would theoretically permit subject identity to be inferred, with potential implications for characterization of the study as non-human subjects research (Moore et al., 2010). Candidate gene studies over a limited number of SNPs carry no such plausible risk of subject identification, and thus may remain feasible, at least for preexisting specimens,
despite the potential for changes in the U.S. regulatory environment that might limit such studies without individual subject consent (Pavlin & Welch, 2015).

For these reasons, studies both of ABCB1 and of other candidate genes that may plausibly mediate MQ neuropharmacokinetics, employing DoDSR specimens as a source of genetic material, may remain the most feasible and efficient means of investigating the pharmacogenetics of MQ neuropsychiatric adverse reactions, in the absence of preferential identification and enrollment of a study population from which appropriate informed consent, and genetic material for whole genome analysis, might be prospectively obtained.

4.5. Conclusions

This study establishes the feasibility and potential utility of conducting future pharmacogenetic studies of neuropsychiatric adverse reactions to MQ among U.S. military populations. Identification of an improved and more specific characterization of the phenotype for MQ neuropsychiatric adverse reactions that could permit cases to be identified retrospectively using electronic medical record data may permit improved candidate gene studies using DoDSR specimens.
Table 4.1. Potential Study Population and Eligible Population From Which Cases and Controls were Selected

<table>
<thead>
<tr>
<th></th>
<th>Potential Study Population*</th>
<th>Mefloquine-Prescribed Population**</th>
<th>Eligible Population***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>% (of total)</td>
<td>n</td>
</tr>
<tr>
<td>All</td>
<td>2,961,927</td>
<td>100</td>
<td>88,219</td>
</tr>
<tr>
<td>Males</td>
<td>2,493,779</td>
<td>84.2</td>
<td>77,894</td>
</tr>
<tr>
<td>Females</td>
<td>468,050</td>
<td>15.8</td>
<td>10,322</td>
</tr>
<tr>
<td>White</td>
<td>2,096,375</td>
<td>70.8</td>
<td>62,857</td>
</tr>
<tr>
<td>Non-White</td>
<td>791,493</td>
<td>26.7</td>
<td>22,627</td>
</tr>
</tbody>
</table>

*Row strata exclude 74,059 individuals with unknown or unspecified self-reported race, and 98 with unknown or unspecified sex.
**Row strata exclude 2,735 individuals with unknown or unspecified self-reported race, and 3 with unknown or unspecified sex.
***Row strata exclude 1,027 individuals with unknown or unspecified self-reported race.

Table 4.2. Eligible Cases and Controls and Final Study Population Case and Control Counts, by Phenotype

<table>
<thead>
<tr>
<th>Eligible Population</th>
<th>Final Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases*</td>
</tr>
<tr>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>All Phenotypes</td>
<td>-</td>
</tr>
<tr>
<td>Suicide</td>
<td>10</td>
</tr>
<tr>
<td>Evacuation</td>
<td>47</td>
</tr>
<tr>
<td>Psychosis</td>
<td>46</td>
</tr>
<tr>
<td>PTSD</td>
<td>493</td>
</tr>
<tr>
<td>Other</td>
<td>4,108</td>
</tr>
</tbody>
</table>

*Without regard to selection as a case or control in another phenotype.

Table 4.3. Sex and Race, Final Study Population, by Case and Control Status

<table>
<thead>
<tr>
<th>Final Study Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>Males</td>
</tr>
<tr>
<td>Females</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Non-White</td>
</tr>
</tbody>
</table>
Table 4.4. ABCB1 rs1128503 Genotyping Results and Hardy-Weinberg Equilibrium Testing, by Case and Control Status and Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Liberal Genotyping</th>
<th>Controls</th>
<th>HWE</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>CT</td>
<td>TT</td>
<td></td>
</tr>
<tr>
<td>All Phenotypes</td>
<td>366</td>
<td>416</td>
<td>138</td>
<td>0.262</td>
</tr>
<tr>
<td>Suicide</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0.573</td>
</tr>
<tr>
<td>Evacuation</td>
<td>19</td>
<td>21</td>
<td>4</td>
<td>0.741</td>
</tr>
<tr>
<td>Psychosis</td>
<td>11</td>
<td>16</td>
<td>5</td>
<td>1.000</td>
</tr>
<tr>
<td>PTSD</td>
<td>160</td>
<td>178</td>
<td>66</td>
<td>0.172</td>
</tr>
<tr>
<td>Other</td>
<td>172</td>
<td>197</td>
<td>61</td>
<td>0.756</td>
</tr>
<tr>
<td></td>
<td>366</td>
<td>416</td>
<td>138</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Note: Excludes 243 subjects not genotyped in the liberal genotyping analysis, and 606 subjects not genotyped in the conservative genotyping analysis. HWE — Hardy–Weinberg equilibrium. PTSD—posttraumatic stress disorder.

Table 4.5. ABCB1 rs2032582 Genotyping Results and Hardy-Weinberg Equilibrium Testing, by Case and Control Status and Phenotype

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Liberal Genotyping</th>
<th>Controls</th>
<th>HWE</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>GT</td>
<td>TT</td>
<td></td>
</tr>
<tr>
<td>All Phenotypes</td>
<td>395</td>
<td>410</td>
<td>135</td>
<td>0.090</td>
</tr>
<tr>
<td>Suicide</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>0.573</td>
</tr>
<tr>
<td>Evacuation</td>
<td>22</td>
<td>17</td>
<td>4</td>
<td>0.724</td>
</tr>
<tr>
<td>Psychosis</td>
<td>11</td>
<td>17</td>
<td>4</td>
<td>0.714</td>
</tr>
<tr>
<td>PTSD</td>
<td>170</td>
<td>177</td>
<td>63</td>
<td>0.138</td>
</tr>
<tr>
<td>Other</td>
<td>188</td>
<td>195</td>
<td>62</td>
<td>0.353</td>
</tr>
<tr>
<td></td>
<td>395</td>
<td>410</td>
<td>135</td>
<td>0.090</td>
</tr>
</tbody>
</table>

Note: Excludes 206 subjects not genotyped in the liberal genotyping analysis, and 675 subjects not genotyped in the conservative genotyping analysis. Values in bold are p<0.05. HWE — Hardy–Weinberg equilibrium. PTSD—posttraumatic stress disorder.

* P<0.05
Table 4.6. ABCB1 rs1045642 Genotyping Results and Hardy-Weinberg Equilibrium Testing, by Case and Control Status and Phenotype

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>HWE</th>
<th></th>
<th>Controls</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-value</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Liberal Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Phenotypes</td>
<td>291</td>
<td>459</td>
<td>191</td>
<td>507</td>
<td>922</td>
</tr>
<tr>
<td>Suicide</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Evacuation</td>
<td>14</td>
<td>20</td>
<td>6</td>
<td>17</td>
<td>42</td>
</tr>
<tr>
<td>Psychosis</td>
<td>8</td>
<td>14</td>
<td>9</td>
<td>20</td>
<td>31</td>
</tr>
<tr>
<td>PTSD</td>
<td>117</td>
<td>210</td>
<td>84</td>
<td>231</td>
<td>419</td>
</tr>
<tr>
<td>Other</td>
<td>151</td>
<td>209</td>
<td>89</td>
<td>232</td>
<td>421</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Conservative Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Phenotypes</td>
<td>246</td>
<td>398</td>
<td>157</td>
<td>441</td>
<td>764</td>
</tr>
<tr>
<td>Suicide</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Evacuation</td>
<td>14</td>
<td>19</td>
<td>4</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>Psychosis</td>
<td>7</td>
<td>10</td>
<td>6</td>
<td>16</td>
<td>27</td>
</tr>
<tr>
<td>PTSD</td>
<td>100</td>
<td>183</td>
<td>75</td>
<td>201</td>
<td>341</td>
</tr>
<tr>
<td>Other</td>
<td>125</td>
<td>180</td>
<td>71</td>
<td>204</td>
<td>353</td>
</tr>
</tbody>
</table>

Note: Excludes 190 subjects not genotyped in the liberal genotyping analysis, and 651 subjects not genotyped in the conservative model. HWE — Hardy–Weinberg equilibrium. PTSD—posttraumatic stress disorder.

Table 4.7. Conditional Logistic Regression of Case Phenotypes with ABCB1 Genotypes Under an Additive Model, Unadjusted P-Values

<table>
<thead>
<tr>
<th></th>
<th>rs1128503</th>
<th>rs2032582</th>
<th>rs1045642</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T vs C</td>
<td>T vs G</td>
<td>T vs C</td>
</tr>
<tr>
<td><strong>Liberal Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.096</td>
<td>0.048*</td>
<td>0.363</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.756</td>
<td>0.883</td>
<td>0.185</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.092</td>
<td>0.093</td>
<td>0.107</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.946</td>
<td>0.567</td>
<td>0.126</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.461</td>
<td>0.186</td>
<td>0.652</td>
</tr>
<tr>
<td>Other</td>
<td>0.256</td>
<td>0.240</td>
<td>0.067</td>
</tr>
<tr>
<td><strong>Conservative Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.284</td>
<td>0.185</td>
<td>0.924</td>
</tr>
<tr>
<td>Suicide</td>
<td>1.000</td>
<td>0.940</td>
<td>0.579</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.216</td>
<td>0.156</td>
<td>0.027</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.299</td>
<td>0.667</td>
<td>0.174</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.568</td>
<td>0.462</td>
<td>0.222</td>
</tr>
<tr>
<td>Other</td>
<td>0.710</td>
<td>0.447</td>
<td>0.325</td>
</tr>
</tbody>
</table>

Note: Values in bold are p<0.05. PTSD—posttraumatic stress disorder.

* P<0.05
Table 4.8. Conditional Logistic Regression of Case Phenotypes with ABCB1 Haplotypes Under an Additive Model, Unadjusted P-Values

<table>
<thead>
<tr>
<th>rs1128503/rs2032582/rs1045642 Haplotype</th>
<th>CTC</th>
<th>CGT</th>
<th>CGC</th>
<th>TTC</th>
<th>TGC</th>
<th>TGT</th>
<th>TTC</th>
<th>TTT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liberal Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.082</td>
<td>0.469</td>
<td>0.193</td>
<td>0.794</td>
<td>0.633</td>
<td>0.845</td>
<td>0.027*</td>
<td>0.271</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.236</td>
<td>0.116</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.642</td>
<td>0.706</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.041*</td>
<td>0.873</td>
<td>-</td>
<td>-</td>
<td>0.774</td>
<td>0.437</td>
<td>0.493</td>
<td>0.087</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.244</td>
<td>0.118</td>
<td>1.000</td>
<td>-</td>
<td>0.249</td>
<td>0.645</td>
<td>1.000</td>
<td>0.556</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.454</td>
<td>0.380</td>
<td>0.041*</td>
<td>0.813</td>
<td>0.886</td>
<td>0.848</td>
<td>0.021*</td>
<td>0.892</td>
</tr>
<tr>
<td>Other</td>
<td>0.103</td>
<td>0.580</td>
<td>0.436</td>
<td>0.424</td>
<td>0.704</td>
<td>0.822</td>
<td>0.217</td>
<td>0.320</td>
</tr>
<tr>
<td><strong>Conservative Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.549</td>
<td>0.524</td>
<td>0.286</td>
<td>0.738</td>
<td>1.000</td>
<td>0.713</td>
<td>0.077</td>
<td>0.606</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.386</td>
<td>0.154</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.644</td>
<td>1.000</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.078</td>
<td>0.703</td>
<td>-</td>
<td>-</td>
<td>0.540</td>
<td>0.704</td>
<td>1.000</td>
<td>0.237</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.662</td>
<td>0.323</td>
<td>-</td>
<td>-</td>
<td>0.125</td>
<td>0.842</td>
<td>-</td>
<td>0.562</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.960</td>
<td>0.469</td>
<td>-</td>
<td>0.942</td>
<td>1.000</td>
<td>0.286</td>
<td>0.113</td>
<td>0.911</td>
</tr>
<tr>
<td>Other</td>
<td>0.529</td>
<td>0.914</td>
<td>0.815</td>
<td>0.791</td>
<td>0.705</td>
<td>0.720</td>
<td>0.255</td>
<td>0.769</td>
</tr>
</tbody>
</table>

Note: Blank cells indicate model could not be run due to collinearity, no in-group variance, or due to maximum likelihood iteration failing to converge. PTSD—posttraumatic stress disorder.

* P<0.05

Table 4.9. Firth’s Penalized Logistic Regression of Case Phenotypes with ABCB1 Genotypes Under an Additive Model, Unadjusted P-Values

<table>
<thead>
<tr>
<th></th>
<th>rs1128503</th>
<th>rs2032582</th>
<th>rs1045642</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T vs C</td>
<td>T vs G</td>
<td>T vs C</td>
</tr>
<tr>
<td><strong>Liberal Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.077</td>
<td>0.032*</td>
<td>0.285</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.703</td>
<td>0.853</td>
<td>0.232</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.073</td>
<td>0.066</td>
<td>0.099</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.810</td>
<td>0.693</td>
<td>0.136</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.451</td>
<td>0.216</td>
<td>0.834</td>
</tr>
<tr>
<td>Other</td>
<td>0.204</td>
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<td>0.069</td>
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<tr>
<td><strong>Conservative Genotyping</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.459</td>
<td>0.251</td>
<td>0.932</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.769</td>
<td>0.746</td>
<td>0.350</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.130</td>
<td>0.167</td>
<td>0.029*</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.883</td>
<td>0.514</td>
<td>0.359</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.775</td>
<td>0.474</td>
<td>0.189</td>
</tr>
<tr>
<td>Other</td>
<td>0.759</td>
<td>0.474</td>
<td>0.403</td>
</tr>
</tbody>
</table>

Note: PTSD—posttraumatic stress disorder.

* P<0.05
Table 4.10. Firth’s Penalized Logistic Regression of Case Phenotypes with ABCB1 Haplotypes Under an Additive Model, Unadjusted P-Values

<table>
<thead>
<tr>
<th>Genotype/Haplotype</th>
<th>CGC</th>
<th>CGT</th>
<th>CTC</th>
<th>CTT</th>
<th>TGC</th>
<th>TGT</th>
<th>TTC</th>
<th>TTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1128503/rs2032582/rs1045642 Haplotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Liberal Genotyping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.062</td>
<td>0.417</td>
<td>0.317</td>
<td>0.692</td>
<td>0.651</td>
<td>0.679</td>
<td>0.026*</td>
<td>0.179</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.334</td>
<td>0.106</td>
<td>0.775</td>
<td>0.775</td>
<td>0.775</td>
<td>0.775</td>
<td>-</td>
<td>0.556</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.030*</td>
<td>0.888</td>
<td>0.215</td>
<td>0.291</td>
<td>0.603</td>
<td>0.709</td>
<td>0.486</td>
<td>0.074</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.403</td>
<td>0.107</td>
<td>0.955</td>
<td>-</td>
<td>0.187</td>
<td>0.468</td>
<td>0.906</td>
<td>0.726</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.599</td>
<td>0.226</td>
<td>0.089</td>
<td>0.720</td>
<td>0.787</td>
<td>0.736</td>
<td>0.025</td>
<td>0.782</td>
</tr>
<tr>
<td>Other</td>
<td>0.053</td>
<td>0.532</td>
<td>0.382</td>
<td>0.329</td>
<td>0.696</td>
<td>0.696</td>
<td>0.234</td>
<td>0.215</td>
</tr>
<tr>
<td>Conservative Genotyping</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.792</td>
<td>0.476</td>
<td>0.348</td>
<td>0.615</td>
<td>0.942</td>
<td>0.648</td>
<td>0.156</td>
<td>0.764</td>
</tr>
<tr>
<td>Suicide</td>
<td>0.615</td>
<td>0.185</td>
<td>-</td>
<td>0.821</td>
<td>0.821</td>
<td>-</td>
<td>0.498</td>
<td>0.990</td>
</tr>
<tr>
<td>Evacuation</td>
<td>0.070</td>
<td>0.547</td>
<td>0.736</td>
<td>-</td>
<td>0.450</td>
<td>0.788</td>
<td>0.133</td>
<td>0.290</td>
</tr>
<tr>
<td>Psychosis</td>
<td>0.348</td>
<td>0.290</td>
<td>-</td>
<td>-</td>
<td>0.225</td>
<td>0.518</td>
<td>0.820</td>
<td>0.924</td>
</tr>
<tr>
<td>PTSD</td>
<td>0.390</td>
<td>0.270</td>
<td>0.384</td>
<td>0.939</td>
<td>0.867</td>
<td>0.203</td>
<td>0.246</td>
<td>0.878</td>
</tr>
<tr>
<td>Other</td>
<td>0.886</td>
<td>0.783</td>
<td>0.935</td>
<td>0.545</td>
<td>0.620</td>
<td>0.482</td>
<td>0.395</td>
<td>0.938</td>
</tr>
</tbody>
</table>

Note: Blank cells indicate model could not be run. PTSD—posttraumatic stress disorder.

* P<0.05

Table 4.11. Association of ABCB1 Genotypes and Haplotypes with PTSD Phenotype for the Full Sample and by Sex

<table>
<thead>
<tr>
<th>Genotype/Haplotype</th>
<th>Study Population Cases Total OR 95%CI</th>
<th>Men Cases Total OR 95%CI</th>
<th>Women Cases Total OR 95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1128503</td>
<td>CT/CC 338 1000 1.0 Reference</td>
<td>272 815 1.0 Reference</td>
<td>66 185 1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>TT 66 199 0.98 0.70-1.39</td>
<td>58 167 1.08 0.74-1.57</td>
<td>8 32 0.58 0.22-1.52</td>
</tr>
<tr>
<td>rs2032582</td>
<td>GT/GG 347 1004 1.0 Reference</td>
<td>279 813 1.0 Reference</td>
<td>68 191 1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>TT 63 203 0.85 0.60-1.20</td>
<td>54 174 0.86 0.59-1.25</td>
<td>9 29 0.80 0.33-1.93</td>
</tr>
<tr>
<td>rs1045642</td>
<td>CT/CC 327 977 1.0 Reference</td>
<td>266 805 1.0 Reference</td>
<td>61 172 1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>TT 84 243 1.09 0.81-1.48</td>
<td>70 198 1.14 0.81-1.60</td>
<td>14 45 0.94 0.46-1.89</td>
</tr>
<tr>
<td>Haplotype</td>
<td>Non-TTT-TTT 364 1078 1.0 Reference</td>
<td>292 876 1.0 Reference</td>
<td>72 202 1.0 Reference</td>
</tr>
<tr>
<td></td>
<td>TTT-TTT 48 144 1.03 0.70-1.53</td>
<td>44 125 1.12 0.74-1.71</td>
<td>4 19 0.59 0.18-1.87</td>
</tr>
</tbody>
</table>

Note: OR by conditional logistic regression. Genotype and haplotype from liberal genotyping analysis.
Figure 4.1. Potential Study Population and Final Eligible Population

Potential Study Population

N=2,961,927

N=2,873,708 excluded for no qualifying prescription for mefloquine

N=88,219

N=23,711 excluded for <12 months prior continuous active duty time

N=64,508

N=18,488 excluded for no medical encounters in each of two prior consecutive 6 month periods

N=46,020

N=304 excluded due to diagnosis of malaria

N=45,716

N=12,929 excluded for no prescriptions in the prior 6 months

N=32,777

Eligible Population
5.1. Summary

This dissertation has presented the results of three studies intended to contribute to improved mefloquine (MQ) pharmacovigilance with the aims of better preventing, detecting, assessing, and understanding neuropsychiatric adverse reactions to the drug.

Use of MQ is associated with a risk of potentially permanent and disabling neuropsychiatric effects (U.S. Food and Drug Administration, 2013), but there is uncertainty in how to best prevent these effects during clinical use of the drug, particularly in prophylaxis. There is also uncertainty in how to detect and assess these more serious effects, particularly in case finding for pharmacoepidemiologic or pharmacogenetic studies. Consequently, the molecular origins of these effects, including any genetic risk factors that may predict such effects, are not yet well studied or understood.

In the Aim 1 analysis, it was determined that there is widespread international agreement in prescribing and patient safety guidance that MQ use be discontinued (DC) at the onset of certain neuropsychiatric reactions that may be considered prodromal to more serious effects. These reactions may be categorized into four standard neuropsychiatric high level group term (HLGT) categories according to the Medical Dictionary for Regulatory Activities (MedDRA) vocabulary. These HLGT categories are anxiety disorders and symptoms, changes in physical activity, depressed mood disorders
and disturbances, and deliria (including confusion). Across six primarily English-speaking countries, prescribing and patient safety guidance documents are in complete agreement that reactions in these HLGTs should prompt the DC of the drug in order to prevent the development of more serious effects. In fewer countries, there is partial agreement that additional reactions in many other HLGT categories, including those within the HLGT category sleep disorders and disturbances, should also prompt DC of the drug or physician consultation (CP).

In the Aim 2 analysis, it was determined that, among reports of adverse reactions to common antimalarial drugs in the FDA Adverse Event Reporting System (FAERS), there exists a distinct syndrome class of more serious neuropsychiatric adverse reactions that is strongly associated with the use of MQ. This syndrome class is defined by a very high probability of reactions within the HLGT category deliria (including confusion), and a moderate probability of other neurologic and psychiatric reactions, including within the HLGT categories dementia and amnestic conditions, and seizures (including subtypes). The characteristic features of this syndrome, including deliria, amnesia and seizure, resemble those of early case reports of serious adverse reactions to MQ that were described at the time as toxic encephalopathy or acute brain (Bernard et al., 1987, 1989; Rouveix et al., 1989). Among cases assigned in latent class analysis (LCA) to this most-likely syndrome class, this syndrome is commonly accompanied by reactions within HLGT categories for which there is either complete international agreement that these should be considered prodromal, including reactions within the HLGTs anxiety disorders and symptoms and depressed mood disorders and disturbances; or partial international agreement these should be considered prodromal and be explicitly listed in physician and
prescribing safety guidance, including reactions within the HLGT sleep disorders and disturbances.

In the Aim 3 analysis, it was determined that the serum and data resources of the U.S. military may be feasibly used to support retrospective pharmacogenetic studies investigating genetic risk factors for adverse reactions to MQ. Although the Aim 3 analysis failed to identify significant associations between polymorphisms in ABCB1 and various surrogate phenotypes consistent with neuropsychiatric adverse reactions to MQ, the analysis nonetheless demonstrated that archived specimens in the DoD Serum Repository (DoDSR) may provide an adequate source of DNA for candidate gene studies, and that these result in marginally acceptable DNA yields and genotyping rates through the use of existing commercial genotyping services. Future investigations using the fully anonymous data and specimens that may be obtained in this manner may be considered IRB-exempt non-human subjects research, and may reduce financial and administrative barriers to such studies. The use of improved diagnostic indicators for the neuropsychiatric syndrome associated with neuropsychiatric reactions to MQ, such as those better informed by the results of the Aim 2 analysis, may permit improved power to confirm putative associations with common polymorphisms in candidate gene ABCB1, as well as adequate power to investigate associations with a limited number of other genetic polymorphisms that may plausibly contribute to population heterogeneity in the drug’s pharmacokinetics.
5.2. Discussion

5.2.1. Strengths and Limitations

This dissertation has a number of strengths. The Aim 1 analysis is the most complete review to date of MQ prescribing and patient safety guidance, and provides a current overview of international regulatory perspectives on the drug. The use of the MedDRA vocabulary in the Aim 1 analysis permitted the standard categorization of the often idiomatic language of drug safety labeling, which permitted the analysis to better explore the degree of consistency in guidance across countries, despite subtle international variation in terminology. The Aim 2 analysis is also the most complete review of reported neuropsychiatric reactions to antimalarial drugs within the FAERS database. As with the Aim 1 analysis, the use of the MedDRA vocabulary in this analysis, and particularly the use of HLGT-level indicators, permitted the standard categorization of reported reactions in a manner that emphasized their common clinical features. The Aim 3 analysis establishes the feasibility of conducting pharmacogenetic studies using the data and serum resources of the U.S. military, and is the first such study to be conducted. Although this study had negative findings, the methods and results may serve as a foundation for future similar studies.

However, these findings must also be considered in light of a number of significant limitations. It is unknown whether MQ safety labeling in non-English speaking countries and in the developing world varies substantially from those in the English-speaking countries examined in the Aim 1 study. A more thorough analysis would have included a review of prescribing and patient safety guidance from non-English speaking countries in the developed world, as well as similar documentation,
where available, from the developing world. Although on the basis of standardization across the European Union (EU) it may be assumed that recent regulatory guidance from the European Medicine Agency (EMA) (European Medicines Agency, 2015) would have resulted in substantially similar recommendations being listed in the DL and MG of EU member nations, this in fact has not been independently verified in this analysis. However, recent editorials in a leading international pharmaceutical journal have alluded to substantially similar recommendations in documentation from other EU member nations (Arznei-Telegramm, 2013, 2016a, 2016b).

Additionally, the Aim 1 analysis was subject to potential miscategorization of reactions owing to the idiomatic characteristics of medical terminology among the English-speaking countries studied. Any expanded analysis would likely be subject to even greater potential for miscategorization, owing to further subtleties in terminology across languages and cultures. The use of the MedDRA vocabulary, which provides for a degree of standardization, including across linguistic translations (Brown et al., 1999), can reduce, but not fully eliminate, such potential miscategorization.

Similarly, owing to the subjective nature of the Aim 1 analysis and the inherent ambiguity in DL and MG language, there may be reasonable disagreement as to whether a particular document should be interpreted as recommending DC or CP at the onset of a particular adverse reaction. For example, in the U.S., the MG acknowledges that it may not be possible for a patient to CP at the onset of certain prodromal reactions, and advises patients, “If you do not have access to a doctor or to another medicine and have to stop taking mefloquine, leave the malaria area and contact a doctor as soon as possible [emphasis added]”. Yet, in the drug safety advisory which announced changes to the DL
and MG, in apparent contradiction to the permissive guidance to DC, the FDA instructs that if certain prodromal symptoms develop, that “it may be necessary to stop mefloquine and take another medication to prevent malaria, but do not do so without first talking with your health care professional [emphasis added]” (U.S. Food and Drug Administration, 2013). This ambiguity suggests further areas of potential improvement in regulatory review of drug safety guidance.

The Aim 2 analysis relied on reported drug adverse event data within the FAERS database. The completeness and accuracy of this data, which for many neuropsychiatric reactions is symptom-based and therefore self-reported, requires the results of this analysis to be interpreted with some caution. In addition to well-described concerns with demographic and suspect drug elements in the database (Getz et al., 2012), these limitations may also result in bias in the reporting of particular reactions by drug, which in the context of LCA likely resulted in the significant differential measurement that eliminated many potentially informative HLGT-level indicators from use in the final LCA model. This was seen in particular with the HLGT-level indicators corresponding to those highly prevalent reactions well-known to be associated with use of MQ, such as those within the HLGTs sleep disorders and disturbances and anxiety disorders and symptoms, and which may consequently have been subject to over-reporting. Although the elimination of these indicators might be considered a strength of this analysis as this elimination resulted in a final model whose indicators in part corresponded to objective and non-self-reported reactions, this differential measurement may have precluded a more complete description of defining features of the neuropsychiatric syndrome. For example, psychotic reactions within the HLGT disturbances in thinking and perception,
were also defining features of early case reports of severe reactions to the drug (Björkman, 1989), but this HLGT-level indicator was excluded from the final LCA model owing to evidence of significant differential measurement.

Although the Aim 3 analysis demonstrated the feasibility of performing retrospective pharmacogenetic studies using DoDSR specimens and U.S. military data, future studies employing these resources may benefit from consideration of the limitations encountered in this analysis. In particular, the considerable loss of subjects in this study through application of the strict criteria intended to minimize inclusion of subjects with potential loss of electronic visibility of medical encounter and prescribing data may be undesirable in future studies. With the exception of the 304 subjects eliminated for a diagnosis of malaria, the application of these criteria resulted in the potential study population being reduced from 88,219 to 32,777. In any future study employing these subjects, particularly those with a case definition employing combinations of diagnoses, and in particular diagnoses corresponding to the Aim 2 syndrome, such restrictions may result in undesirably low case counts.

Similarly, given the linear association of genotyping call rates with DNA yield, and the higher average DNA yield of the 1 ml aliquots than the 0.5 ml aliquots in the pilot study, future studies may wish — subject to AFHSB permission — to consider requesting larger volumes of serum than the standard 0.5 ml DoDSR aliquot. Given the variability in DNA yield demonstrated in both the pilot and substantive study, and the availability of multiple specimens per individual, it may be appropriate to consider requesting such larger volumes by pooling serum from multiple specimens collected over time, rather than from a single specimen.
5.2.2. Public Health Significance

The results of this dissertation have potential public health significance and may serve to inform regulatory reevaluation of MQ in certain countries. In the Aim 1 analysis, it was found that there is only partial international agreement that reactions within the HLG T sleep disorders and disturbances should prompt CP or DC of the drug, as determined by corresponding medication guide (MG) and drug label (DL) guidance. The Aim 1 analysis found corresponding MG and DL guidance to this effect in the patient and prescribing guidance documents of only 2 of the 6 (33%) countries studied. The analysis also found significant variation in the content of such guidance between countries — ranging from guidance in Canada that bad dreams are “usually mild” and “do not cause people to stop taking the medicine”, to guidance in the United Kingdom and Ireland that abnormal dreams should prompt the immediate DC of the medication. Similarly, the Aim 2 analysis identified that reactions within the HLG T sleep disorders and disturbances, which include abnormal dreams, nightmares, and insomnia, are commonly reported (in 8.0%, 7.6%, and 16.5%, respectively) among cases assigned by the final HLG T-level LCA model to the most-likely neuropsychiatric syndrome class.

Abnormal dreams and insomnia have long been known to be associated with MQ use (Boudreau et al., 1993), and were clearly identified during early post-marketing studies as being more strongly associated with use of MQ than with other antimalarials. However, in the U.S. and a number of other countries, including those studied in the Aim 1 analysis (Nevin & Byrd, 2016), such reactions have not been explicitly described by drug regulators to be prodromal to more serious effects. International and U.S. drug
regulators may wish to consider the evidence presented in this dissertation, as well as
from recent case reports (Livezey et al., 2016; Nevin, 2012a; Peterson et al., 2011) in
considering revisions to the MQ DL and MG. Based on this evidence, a precautionary
approach to MQ pharmacovigilance would explicitly recommend the immediate
discontinuation of the drug, and its replacement with an alternative antimalarial
medication, at the onset of any reaction within the HLGT sleep disorders and
disturbances.

5.2.3. Future Research

As awareness grows of the potential for chronic neuropsychiatric effects from
MQ, including among military personnel and veterans, and for these to confound the
diagnosis of other prevalent neuropsychiatric conditions (Livezey et al., 2016), there may
be interest in performing additional pharmacoepidemiological and pharmacogenetic
studies to better characterize the epidemiology and risk factors for these effects (Nevin &
Ritchie, 2016).

The feasibility of performing retrospective pharmacogenetic studies using the
serum and data resources of the U.S. military, as demonstrated in the Aim 3 analysis,
suggests opportunities for future research in this area. For example, a revision to the Aim
3 study, using improved diagnostic surrogates, may be considered in light of the Aim 2
study results. The use of a potentially more specific diagnostic surrogate, to include
electronic diagnoses of confusion, amnesia, or seizure, or combinations thereof, together
with other diagnoses consistent with the Aim 2 neuropsychiatric syndrome, may provide
improved specificity and hence increased power to detect potential genotype-phenotype
associations in retrospective pharmacogenetic studies. Although such combination of
diagnoses may risk being confounded by other conditions prevalent in deployment settings, particularly traumatic brain injury, the use of additional available U.S. military data resources, including post-deployment health assessments, may permit minimizing the number of subjects with a reported history of such potentially confounding conditions. Although candidate gene ABCB1 may remain of interest in such future studies given existing evidence in favor of such an association, other plausible candidate genes, in particular methylenetetrahydrofolate reductase (MTHFR), have been proposed as potentially mediating risk of more serious neuropsychiatric adverse reactions to MQ (Livezey et al., 2016). In this respect, it is intriguing that the prior case-control study that used DoDSR specimens as a source of genetic information identified polymorphisms in MTHFR as being associated with an increased risk of epilepsy in a U.S. military population (Scher et al., 2011).

5.3. Conclusions

This dissertation has explored three specific aims related to improving the pharmacovigilance of neuropsychiatric adverse reactions to MQ. The studies presented in this dissertation suggest that more serious adverse effects from MQ may be better prevented through improved recognition of prodromal symptoms, as identified in international drug labeling; that more serious adverse effects may be better assessed and detected through appreciation of the distinct and specific syndrome of neuropsychiatric adverse reactions to the drug, as identified through LCA of FAERS data; and that neuropsychiatric adverse effects from the drug may be better understood through future
retrospective pharmacogenetic studies informed by the results of this LCA, and made more feasible through the use of existing U.S. military data and serum resources.
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Military Service

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Posters

2014 Nevin RL. Historical insights into the neurotoxicity of the 8-aminoquinolines: Implications for the development of tafenoquine and for global malaria control efforts. Poster presented at: Johns Hopkins 2014 World Malaria Day Conference; April 25, 2014; Baltimore, MD.


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