

A case-control study to identify risk factors for myocarditis with clozapine

1. Aims

This project aims to identify risk factors for clozapine-induced myocarditis. This grant application relates specifically to the investigation of possible genetic predispositions, which might allow patients at risk of myocarditis to be identified before commencing clozapine.

Hypothesis

A predisposition to clozapine-related myocarditis is conferred by genetic polymorphism in the human leukocyte antigen region (HLA) of the DNA.

2. Background

With the announcement in December 2007 by the US FDA that all individuals of Asian ancestry should undergo genetic testing for HLA-B*1502 prior to commencing treatment with carbamazepine, genetic screening for a drug hypersensitivity reaction was recognised by a drug regulatory authority for the first time.¹ HLA-B*1502 predisposes to Stevens Johnson syndrome or toxic epidermal necrolysis with carbamazepine and is found exclusively in Asians.^{2,3} More recently, in February 2008, the first randomised controlled trial demonstrating the predictive value of a genetic predisposition for a drug hypersensitivity reaction was published.⁴ The drug reaction was hypersensitivity with abacavir and the genetic polymorphism was HLA-B*5701. All of those with immunologically confirmed hypersensitivity were subsequently found to have HLA-B*5701.

Against this background of the demonstrated worth of the genetic investigation of hypersensitivity reactions, we propose to search for a genetic marker for hypersensitivity myocarditis with clozapine. This project is part of a research program designed to improve the safe use of important drugs, involving the use of case-control studies to identify subgroups of individuals at high risk of serious adverse drug effects.

The program makes use of the effective spontaneous monitoring schemes in Australia and New Zealand which accumulate reports of significant adverse drug reactions submitted by medical practitioners, pharmacists and pharmaceutical companies. The number of cases of specific drug reactions reported in Australia has often been greater than in other advanced countries and the cases therefore form a valuable source of cases for epidemiological studies. The methodology has been used successfully by the current chief investigator (CIA) to study a series of significant adverse drug reactions involving flucloxacillin,⁵ tiaprofenic acid⁶ and aspirin.⁷

Using these two spontaneous reporting schemes, we have a starting point of over 200 cases of myocarditis with clozapine, most of which are well documented. It is unlikely that researchers elsewhere will have access to a comparably large number of well-documented cases.

The expected outcome and long term benefits of the present study are:

- The identification a single nucleotide polymorphism associated with a strong

- predisposition for the development of myocarditis with clozapine;
- The identification of phenotypic characteristics which may modify the genetic predisposition to either increase or decrease the risk;
- The prospect of clozapine, the most effective medicine for treatment-resistant schizophrenia, being made more widely available to those most likely to benefit; and
- The burden of schizophrenia to patients, family members, friends and wider society being reduced through those affected becoming contributing members of the community.

In addition, the study has the potential to make a scientific contribution by increasing understanding of:

- The characteristics and natural history of clozapine-associated myocarditis; and
- The genotypic and phenotypic basis of hypersensitivity reactions.

2.1 The nature of the problem

Clozapine is recommended by the Royal Australian and New Zealand College of Psychiatrists as the agent of choice in schizophrenia when two or more other therapies have failed, a situation which occurs in around 30% of patients.⁸ Those in whom it is effective without significant adverse effects may recover to a functional life. Clinical trial data indicate that it is more effective than other antipsychotic agents.^{9 10} Further the InterSePT trial demonstrated a significant reduction in suicide rate with clozapine compared with another widely used atypical antipsychotic, olanzapine.¹¹

Most use of clozapine is not subsidized through the Pharmaceutical Benefits Scheme, but information from the two Australian sponsors, Novartis and Hospira, suggests that over 10,000 Australian patients are currently taking clozapine.

Despite its efficacy, the use of clozapine has been restricted to the most severely affected patients because of life threatening adverse effects including agranulocytosis and myocarditis. Patients taking clozapine are monitored in a mandatory scheme to detect the onset of agranulocytosis at an early stage in its evolution. A non-mandatory protocol for monitoring for myocarditis was promulgated by the sponsor, Novartis, in December 1999 in Australia (but not New Zealand).

From 1993 to the end of 2007, the Australian Adverse Drug Reactions Advisory Committee (ADRAC) received 226 reports of myocarditis with clozapine (91% reported since 1999). In addition to 10 fatal cases in this dataset, four fatalities occurring between 2004 and 2006 have been identified from the National Coroners' Information Service (NCIS) database and the Department of Forensic Medicine, NSW. In New Zealand, 26 reports have been recorded by the Intensive Medicine Monitoring Programme (IMMP) which is a nationwide program and part of the New Zealand Pharmacovigilance Centre (NZPhVC). Several adverse drug reactions bulletin articles have been published in both countries alerting medical practitioners to the risk of clozapine-induced myocarditis.

The incidence of clozapine-induced myocarditis is uncertain. On the basis of Australian cases reported to ADRAC up to early 1999, Kilian et al proposed a minimum incidence of 1 in 500 clozapine recipients.¹² In another Australian case

series published in 2004, the incidence was 8.5%, based on eight cases from a cohort of 94 patients started on clozapine.¹³ This result is unexpectedly high, but the methodology was more robust than that used for earlier estimates. Another estimate from a health service in the Hunter Valley put the incidence at more than 2%, and higher than that for agranulocytosis.¹⁴ Probably because of different levels of awareness of the condition, no other country has recorded an incidence as high as that found in Australia.

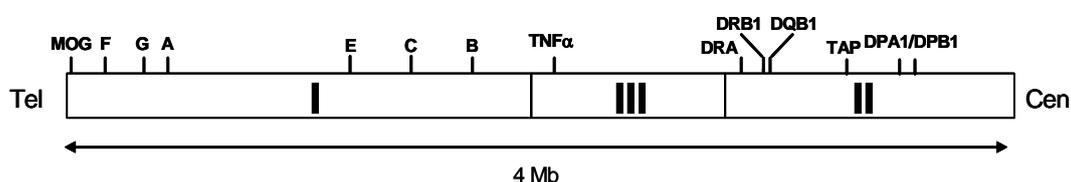
The clinical presentation of clozapine myocarditis has ranged from a fulminant onset with death within hours, to a mild influenza-like illness where the presence of myocarditis is signified by the onset of tachycardia and increased troponin I and/or T. A high level of diagnostic suspicion is needed since sudden death in patients with drug-resistant schizo-phrenia may otherwise be attributed to suicide, drug-induced arrhythmia, drug overdose or other unknown cause^{15 16} while the features, such as fever,¹⁷ tachycardia and eosinophilia,^{18 19} commonly resemble the symptom complex that accompanies the initiation of dosing with clozapine.

Although there are many publications describing individual cases of myocarditis^{17 20 21} as well as articles reviewing cases reported to a national adverse drug reactions reporting program,^{12 22 23} to date no systematic studies of this important adverse reaction have been conducted. This deficiency means the evidence base for monitoring and diagnostic guidelines is poorly-informed, and nothing is known of the risk factors for clozapine-induced myocarditis.

The mandatory monitoring for agranulocytosis has led to the employment of ‘clozapine coordinators’ in the major psychiatric units in Australia whose role is to ensure compliance with the weekly full blood monitoring, and the more recently implemented cardiac protocol. Concern around tolerability in the early phase of treatment has resulted in a standardised approach to clozapine initiation with careful documentation while the patient is hospitalised. This situation together with the large number of cases reported to ADRAC, present an opportunity for Australia to make a unique and significant contribution to the treatment of schizophrenia by investigating the clinical history and risk factors for clozapine-related myocarditis. Including New Zealand data will strengthen the study.

2.2 Pharmacogenetics of drug hypersensitivity reactions

Clozapine-related myocarditis is recognised to be a drug hypersensitivity reaction.²⁰ The region of DNA most profitably studied in relation to hypersensitivity reactions has been the human leukocyte antigen (HLA) complex on chromosome 6p21, spanning 4 MB and containing 140 expressed genes, of which about 40% are involved in immune function.²⁴ At the telomeric end of the HLA complex is the class I region containing six expressed HLA genes (HLA-A, B, C, E, F and G), proceeding towards the centromere, the class III region spans ~0.7 Mb and contains the TNF locus, while the class II contains HLA-D genes (Figure).



Recent investigations have found strong associations between a range of drug hypersensitivity reactions and polymorphisms on HLA-B, a region with more than 800 known variants.²⁷

In one of the most widely quoted of these studies, 100% of Han Chinese developing Stevens Johnson syndrome with carbamazepine² were found to have HLA-B*1502, while this polymorphism was present in only 3% of controls. This association was the subject of the FDA announcement in December 2007.¹ In contrast to the Han Chinese, Caucasians with carbamazepine-associated Stevens Johnson syndrome did not possess the HLA-B*1502 variant,³ but protection against any hypersensitivity with carbamazepine appeared to be conferred by the presence of HLA-B*0801, which was found in all those without this condition.²⁸

Another genetic association with a high positive predictive value is HLA-B*5801 with severe cutaneous reactions with allopurinol.²⁹ 100% of those with the adverse effect had the variant and 15% of the control subjects.

The randomised controlled trial mentioned above (section 2) built on retrospective case-control studies,³⁰ similar to the one proposed here, showing a strong association between abacavir hypersensitivity and HLA-B*5701.⁴ While, all those with immunologically confirmed hypersensitivity had the polymorphism, HLA-B*5701, a subgroup with HLA-B*5701 were able to take abacavir without adverse effect. The reason for the safe use in this group was unclear, but it may have been related to genetic or environmental factors. In this study HLA-B*5701 had a negative predictive value of 100% and a positive predictive value of 48%.

The above study illustrates that a single genetic predisposition for a hypersensitivity reactions may not be a sufficient condition. Hence, consideration that there may be more than one predisposing polymorphism and attention to phenotypic and environmental characteristics are essential and may have unforeseen benefits. Besides documentation of these factors, the success of genetic studies depends on the diagnostic precision of cases, careful definition of the control group to exclude potential cases, and the quality and resolution of the genetic analysis.^{27 31} In addition, association with genetic polymorphisms may be dependent on the severity of the reaction (e.g. SNP on TNF α gene linked to severe but not mild carbamazepine-induced rash³²). Hence, the characteristics of the cases will be factored into the genetic analysis, specifically presence of eosinophilia and rises in creatine kinase, and degree of increase in troponin I/T and C-reactive protein.

2.3 *Phenotypic and environmental risk factors*

The rate of dose titration and metabolic impairment have been proposed as risk factors, but without data to support the proposal.³³ In addition to genetic factors, our study will investigate a broad range of intrinsic and extrinsic factors, including age, gender, race, body mass index, ability to metabolise clozapine, rate of dose titration and concomitant medication and concurrent illnesses (including atopic illness and chronic viral infection).

2.4 *Current study progress*

Approvals have now been received from 11 site-related ethics committees, including an approval which applies to all of NSW, and 2 further applications have been submitted. One of these approvals also covers access to samples from individuals who died of clozapine-related myocarditis and held by the Department of Forensic Medicine, NSW. Similar permission is being sought for samples held by the Victorian Institute of Forensic Medicine. Contacts have been made in preparation for further ethics applications for South and Western Australia in the near future.

There are currently 112 patients documented in the study database including 30 confirmed cases and 49 confirmed controls. The other 33 patients are either failed cases/controls or incompletely documented. These cases and controls were documented through visits to over 20 sites, with visits to more than one site required for some individuals. Of the 30 confirmed cases 23 had been reported to ADRAC, 6 were notified to the researchers by local staff, and one was located by a search of the NCIS database.

Because of the special circumstances controlling the initiation of clozapine as described in section 2.1 above, it has been possible to document daily data on medication, symptoms and vital signs along with pathology results determined weekly or more frequently. Data on ethnicity and prior medical history require further follow-up in some instances, but the clinical history from the commencement of clozapine has been fully documented in almost all cases and controls.

The 30 confirmed cases are 22 men and 8 women aged 22-74 (mean 37) year. Onset was during the third week of clozapine therapy for 28 cases. The current guidelines do not recommend monitoring for myocarditis beyond 14 days.

The phenotypic characteristics of the confirmed cases of myocarditis are diverse, but this does not exclude there being a single genetic predisposition. Myocarditis from any cause is known not be homogeneous in its manifestations. Further, the clinical characteristics may reflect environmental and non-genetic host factors. A paper is currently being prepared for publication on the confirmed cases, which will serve as an evidence base for guidelines for monitoring clozapine patients for myocarditis.

With regard to the suggestion by Devarajan et al³³ that myocarditis may be associated with an inability to metabolise clozapine, data on clozapine and norclozapine plasma concentrations have been available for 3 confirmed cases to date and the data gave no indication of metabolic impairment. Further analyses, including the effect of rate of dose titration as a possible risk factor, require a larger dataset and will be undertaken as funding allows. The collection of blood and saliva and extraction of DNA from confirmed cases and controls will commence shortly.

3. Research plan – Methods and techniques to be used

3.1 Case definition

A case definition for clozapine-induced myocarditis has been established by an expert panel from an analysis of cases reported to ADRAC. It has been further refined on

the basis of the first 30 confirmed cases and has been accepted by the study's steering group.

Onset of new symptoms within 45 days of commencing clozapine **PLUS**

- Histological evidence of myocarditis at post-mortem or on myocardial biopsy in the absence of other plausible explanations including *confirmed* viral infection or *exposure* to other likely causative agents within an appropriate timeframe. **OR**
 - New signs of cardiac dysfunction (e.g. persisting tachycardia, third heart sound, basal crepitations, peripheral oedema) with, or without, febrile systemic illness **PLUS**
 - At least ONE of the following diagnostic abnormalities with or without significant eosinophilia (≥ 2 ULN):
 - Elevated troponin-I and/or -T levels (≥ 2 x upper limit of normal - ULN)
 - Elevated CK-MB levels (≥ 2 ULN)
 - Evolutionary electrocardiographic changes (involving ≥ 1 mm ST segment depression or T wave inversion in two or more contiguous leads, excluding lead avR) consistent with myocarditis, with no other obvious cause
 - chest X-ray evidence of heart failure
 - evidence of left or right ventricular systolic dysfunction by echocardiogram, gated pool blood scan, magnetic resonance imaging or contrast ventriculogram
 - MRI diagnostic of myocarditis
- In the absence of alternative plausible aetiologies:
- i. *Confirmed* viral infection
 - ii. *Exposure* to other likely causative agents within an appropriate timeframe.
 - iii. No other likely explanation for these findings, including acute myocardial infarction, neuroleptic malignant syndrome, pulmonary infection or embolism and severe sepsis.

The definition has been made more specific than might be appropriate for establishing a clinical diagnosis, to avoid contaminating the case series with 'non-cases' which would substantially reduce the statistical power of the study.

3.2 *Controls*

To maximize statistical power, four controls are to be matched to each case. Controls will be selected from among the patients who initiated treatment with clozapine at the same inpatient unit, and will be chosen in a systematic fashion from those whose treatment commenced closest in time to the cases. Controls, in addition, will have been treated with clozapine for at least 45 days without developing myocarditis.

3.3 *Recruitment and data collection*

The sources of cases reported to ADRAC and the New Zealand IMMP will be the guide as to which psychiatric units to approach to seek their involvement and obtain

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local ethics committee approval. In addition the National Coroners' Information Service database will be searched for cases not notified to ADRAC.

Step 1 – case identification

With the help of local staff (e.g. clozapine coordinator), cases of possible myocarditis reported to ADRAC will be identified along with any additional not reported. All documentation in the medical records required for the study (including pathology results) will be abstracted and data relevant to the diagnosis of myocarditis will be reviewed by the steering group for the study (includes a cardiologist) for compliance with the case definition.

Step 2 – control identification

Again with the help of local staff, records documenting prescribing of clozapine will be searched for potential controls. The controls will have been treated for at least 45 days without developing myocarditis (as confirmed by pathology and cardiology results). The medical records of the controls will be consulted and full data extraction will occur.

Step 3 – Data verification

Efforts will be taken to verify data obtained from medical records and to fill complete information on ethnicity, smoking status and atopic disease. Sources used will be the clozapine coordinator, treating psychiatrist and the patient themselves or next of kin, depending on the preference of the unit and the health of the patient.

Step 4 – blood or saliva samples

For the cases or controls currently under the care of a recruited psychiatric unit, the permission of the head of the unit and/or the treating psychiatrist will be sought to contact individual patients to seek their consent for provision of a blood or saliva sample for extraction of DNA.

Budget for visiting sites

Visiting sites in Victoria is relatively inexpensive, but documenting cases and controls in other Australian states and in New Zealand will require costs for airfares, accommodation, local travel and meals. Experience has indicated that a 5-day interstate visit at budget rates costs around \$900. At maximum efficiency (no time lost in travel or wasted because of mis-placed files), 10 potential cases can be documented in 5 days. In a similar visit, 15 controls could be documented. The estimated total cost to document interstate is then for cases \$18,000 (200 potential cases) and controls \$28,800 (480 controls for 120 confirmed cases). An estimated 10 further visits would be required to collect samples at a cost of \$9,000.

Most of the New Zealand cases could be documented in a single 2-week visit at an estimated total cost of \$2,600. Three further similar visits would be required to document the controls and conduct sample collection for an estimated total cost of \$7,800.

3.4 Analysis

We expect to obtain data for around 200 cases of myocarditis. With a 4:1 control:case ratio, 80% power and a two sided alpha of 0.05, in a logistic regression model, we

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will be able to detect an odds ratio of 2.4 for a risk factor with 5% prevalence in the controls. Similarly we will be able to detect odds ratios of 1.9-1.6 for risk factors with control prevalence of 10-50%. For multivariate analysis, allowing for 20% missing data and adjustment for two variables in multiple logistic regression (using the rule of thumb of inflating required sample size by 10% for each covariate), we have 80% power to detect an odds ratio of 2.2 for a risk factor with control prevalence of 10%.

Case and control data will be analysed using logistic regression to assess the evidence for association of possible risk factors (phenotypes). While controls will be matched by psychiatric unit and loosely matched for time of commencement of clozapine, we do not propose to employ conditional logistic regression to account for this matching since neither of the matching factors is anticipated to be strongly associated with risk of myocarditis. Age and sex are likely risk factors although the extent of their effect is unclear; so cases and controls are not matched on either of these characteristics. All potential risk factors will be analysed in univariate analyses. Each potential risk factor will subsequently be analysed in a multivariate logistic regression adjusting for age and sex.

Dr Rubio (AI; Rubio is unable to be a CI because of his other grant applications) is the genetic investigator on the project with 8 years' experience in HLA genetics. On his advice, for the genetic analysis, we will conduct exploratory mapping of variation in the HLA complex by typing the HLA-A, B and DRB1 genes at low (two-digit) resolution in a subset of 100 cases and 400 controls matched for ethnicity to look for main effects. The benefits offered by using this approach are two-fold: 1) these HLA genes are well-spaced across the HLA complex 2) they are highly polymorphic. This mapping approach will therefore provide us the opportunity to map a causal variation to a particular region of the HLA complex and will be sensitive enough to interrogate numerous HLA haplotypes. The region showing the strongest signal (difference between cases and controls) will be sequence-typed at high resolution to search for single polymorphisms occurring at statistically significantly different rates between cases and controls.

The recommendation of Dr Rubio is that the best option at present for these analyses is Illumina, San Diego, who use MHC SNP panels containing 2,360 SNPs which are tailored specifically for disease association studies on the HLA complex. This company currently charge US\$159 per sample to conduct both exploratory and fine mapping. However, if a suitable facility opens in Australia we will use this.

Only risk factors, whether they be genotypic, phenotypic or environmental, associated with high odds ratios will be of clinical value. The study will be adequately powered to identify such factors. The intended number of cases for the genetic component of this study exceeds those in previous successful studies in which exploratory mapping led to identification of single genetic markers for a hypersensitivity reaction: the study of Stevens-Johnson syndrome with carbamazepine included 44 cases and 101 controls;² a cohort of 200 patients of whom 18 developed the hypersensitivity was included in the study of hypersensitivity with abacavir.³⁴

3.5 *Bias, confounding and quality control*

As with any case control study, it will be necessary to avoid predictable sources of bias and confounding. In particular:

- The risk that information may be differentially extracted from patient notes for cases and controls (particularly as a result of knowledge of case or control status) will be addressed through prescriptive guidelines in a procedure manual. It will not be practicable to conceal the development of myocarditis from the data extractor.
- The risk that the cases and controls may differ in their medical care, record keeping or the intensity of the diagnostic process in seeking to establish a diagnosis of myocarditis will be reduced by matching case and control groups from the same treating unit, within a close time proximity. The quality and completeness of data will be improved by interview of the patient or a surrogate, where this is possible.
- The case series may not be representative of all cases of clozapine induced myocarditis because only those cases sufficiently severe to meet the case definition will be included, and because there may be low case ascertainment of fatal cases due to failure to fully investigate the cause of death. However this is unavoidable because of the need to ensure that only true cases are included within the case series. The consequence may be that the results are strictly generalisable only to the more severe, non-fatal, cases.
- The control series might include unidentified cases of myocarditis, thereby reducing the power of the study. However, this likelihood is reduced by the cardiac monitoring protocol designed to detect myocarditis and by the requirement that all controls be treated with clozapine for at least 45 days. Also features developing during clozapine initiation that are associated with myocarditis are being documented in the controls so that the presence of these factors can be considered in the analysis.

4. **Outcomes and significance**

The primary purpose of this study is to provide information that will allow clozapine to be used more safely and therefore to make it available to a broader range of patients who may benefit from its superior efficacy in the treatment of schizophrenia.

Previous investigations of drug hypersensitivity reactions indicate that the identification of a single allelic variant to which more than 90% of cases of myocarditis are attributable is a realistic expectation.²⁷ We may also find that other factors act on the genetic predisposition to modify the risk. These factors may include rate of dose titration or concurrent medication.

With this information, it will be possible to develop a pre-clozapine genetic screening test to identify those at high risk of myocarditis. Psychiatrists will then be able to prescribe clozapine with confidence to those at low risk. It is also conceivable that information on risk modifying factors may indicate a way that clozapine can be

initiated safely for those at high risk, so that myocarditis can be avoided in this group as well.

Whatever the precise results of the study, the anticipated outcome is that more schizophrenic patients will be able to benefit from clozapine enabling them to lead productive lives in the community, with the burden of the illness relieved for themselves, their families and friends. Even prior to the recent publication of the HLA-B*5701 screening trial,⁴ the prescription of abacavir in the United Kingdom increased as a result of identification of the allele predisposing to hypersensitivity.³¹

Beyond the clozapine-specific outcomes, the study with its combination of investigation of symptomatic features, pathology results, cardiology investigations, environmental factors and genetic analysis has the potential to become a landmark study in the understanding of drug hypersensitivity reactions, particularly those targeting organs other than the skin with or without eosinophilia. The potential of the study to include data on as many as 200 cases and obtain comprehensive documentation of the adverse reaction far exceeds the capacity of most studies of hypersensitivity reactions and will greatly enhance the power of the study.

If the study results are negative, meaning that no risk factors, genotypic, phenotypic or environmental, are identified at a statistically significant level, the study will have reliably eliminated several areas for investigation of this adverse reaction, and indicated areas warranting further research. It will also have comprehensively characterised clozapine-related myocarditis and its clinical history, which will have considerable value in itself.

Myocarditis with clozapine is arguably the most important adverse drug reaction issue in Australia at present and is in urgent need of adequate investigation. The study has the potential to make a valuable and unique contribution to the practice of psychiatry worldwide, and also to pharmacovigilance and pharmacogenetics, employing the outstanding pharmacovigilance programs in Australia and New Zealand as its basis.

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