Examining the potential preventative effects of minocycline prescribed for acne on the incidence of severe mental illnesses: a historical cohort study

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Abstract:

Background: Animal studies suggest that the antibiotic and microglial activation inhibitor, minocycline, is likely to have a protective effect against the emergence of psychosis but evidence from human studies is lacking. The aim of this study is to examine the effects of exposure to minocycline during adolescence on the later incidence of severe mental illness (SMI).

Methods: A historical cohort study using electronic primary care data was conducted to assess the association between exposure to minocycline during adolescence and incidence of SMI. The Incidence Rate Ratio (IRR) was measured using Poisson regression adjusted for age, gender, time of exposure, socioeconomic deprivation status, calendar year and co-medications.

Results: Early minocycline prescription (n=13 248) did not affect the incidence of SMI compared with non-prescription of minocycline (n=14 393), regardless of gender or whether or not the data were filtered according to a minimum exposure period (minimum period - IRR 0.96; 95% CI 0.68-1.36; p=0.821; no minimum period - IRR 1.08; 95% CI 0.83-1.42; p=0.566).

Conclusions: Exposure to minocycline for acne treatment during adolescence appears to have no effect on the incidence of SMI.

Key words: schizophrenia/drug repurposing/preventive effect/tetracycline

Introduction

There is accumulating evidence to suggest that neuroinflammation arising from activation of microglia plays an important role in the aetiology of severe mental illnesses (SMI) such as schizophrenia, bipolar depression, or other psychotic conditions (Takahashi et al., 2016). It has been proposed that the therapeutic effect of atypical antipsychotics may be related to the reduction of the microglial inflammatory response (Monji et al., 2013), fuelling the hypothesis that control of microglial activation could provide a promising therapeutic strategy for management of psychosis (Keller et al., 2013). The second generation tetracycline minocycline, a known inhibitor of microglial activation (Zhang and Zhao, 2014), has been postulated as a promising adjuvant therapy to antipsychotics and its potential beneficial effects have been assessed in various clinical trials (Chaudhry et al., 2012; Chaves et al., 2015; Ghanizadeh et al., 2014; Husain et al., 2017; Kelly et al., 2015; Khodaie-Ardakani et al., 2014; Levkovitz et al., 2010; Liu et al., 2014; Miyaoka et al., 2012). These clinical trials were conducted using very different study designs, however, and the results differed considerably (Oya et al., 2014). Although they showed evidence of a beneficial effect of adjuvant therapy with minocycline in negative symptoms of schizophrenia and related disorders, the small populations and short lengths of study periods were common limitations (Xiang et al., 2016). A preventative effect of minocycline – i.e., exposure before the onset of the disease – has also been hypothesised but tested only in animal

models (Levkovitz et al., 2007; Monte et al., 2013; Zhang et al., 2007). Recently, Giovanoli et al. (2016) postulated that attenuation of inflammatory reactions in peri-pubertal stress exposure could prevent the adult onset of behavioural pathologies and conducted investigations to determine whether pre-symptomatic administration of minocycline could prevent the emergence of behavioural abnormalities, concluding that minocycline had a preventative effect on sensorimotor gating and psychosomatic drug sensitivity in animals but failed to prevent the stress-induced increase in anxiety-like behaviour. A preventative effect of minocycline, however, has not been evaluated in clinical or epidemiological studies. In past years, minocycline was widely used as the first line oral antibiotic for treatment of acne, but its use over the last decade has fallen due to safety and cost concerns (Garner et al., 2003). Although minocycline's use to treat acne is not recommended by NICE Clinical Knowledge Summaries (National Institute for Health and Care Excellence, 2014), it is still used primarily for this indication (National Institute for Health and Care Excellence, 2015) which usually starts in adolescence and frequently resolves by early twenties, being most prevalent in individuals aged 15-24 years (Zaenglein et al., 2016). Here, we present the first historical cohort study using primary care electronic data to investigate whether exposure to minocycline during adolescence in individuals diagnosed with acne is associated with a reduced incidence of SMI.

Methods

Study Design and Population

A historical cohort study was conducted using data from the Clinical Practice Research Datalink database (CPRD; https://www.cprd.com), a well-established United Kingdom (UK) primary care research database of electronic health records (García Rodríguez and Pérez Gutthann, 2002; Herrett et al., 2010). As primary care is free at the point of delivery, it has almost universal coverage in the UK. The database has been described in detail elsewhere (Williams et al., 2012).

The study protocol was reviewed and approved by the Independent Scientific Advisory Committee (ISAC) for Medicines and Healthcare products Regulatory Agency (MHRA) Database Research (ISAC protocol number 15_245A2, June 2016).

Selection of Minocycline Users and the Comparison Group

The study population comprised those patients with one or several recorded diagnoses of acne (Supplementary Table 1) at the ages of 15 to 20 years old (inclusive) during the years 1991 to 2005. To avoid potential bias from missing data, only patients with twelve or more months registration before a diagnosis of acne in the CPRD were included. The study population was divided into an exposed cohort (those individuals treated with oral minocycline) and a control cohort (those individuals prescribed oral acne antibiotics for moderate or severe

acne other than minocycline: lymecycline, tetracycline, oxytetracycline or erythromycin). Exposure was identified as recorded prescriptions of these drugs in the CPRD database (Supplementary Tables $2_{a,b}$). Patients with a recorded prescription of any of these drugs before the diagnosis of acne were not included in the study.

The start of the follow-up period for all patients was the date of the first completion of a minimum continuous treatment with minocycline or alternative treatment (Figure 1). Patients with any recorded prescription of isotretinoin – a retinoid used to treat moderate and severe acne that has been linked to adverse psychiatric reactions in several reports (Ludot et al., 2015) – or any recorded prescription of an antipsychotic drug prior to the start of follow-up were not included in the study.

[Insert Figure 1]

Figure 1. Example of the follow-up period of a patient exposed to minocycline (exposed cohort) in the primary and sensitivity analyses

The follow-up period ended at the earliest of the following: the date the patient left the practice, death date, the latest date of data collection (January 2016), or the earliest date of a diagnosis of SMI (outcome). Individuals prescribed an alternative treatment as first-line option were considered controls until they were

prescribed minocycline, when the follow-up as control individuals was stopped. They switched to the exposed cohort if a minimum treatment with minocycline was completed (Figure 2). For patients who were prescribed an alternative treatment after being exposed to minocycline, follow-up was continued in the exposed cohort. All individuals were censored if they had a recorded prescription of isotretinoin after the start of follow-up date.

[Insert Figure 2]

Figure 2. Example of the end of follow-up as a control cohort individual and the start of follow-up period as an exposed cohort individual in the primary and sensitivity analyses. In the primary analysis, the date of the start of follow-up is the completion of a minimum duration of treatment with an alternative drug or minocycline and the dates of the end of follow-up as a control individual and the start of follow-up as an exposed individual cannot overlap. In the sensitivity analysis, there is not a minimum duration of treatment and the dates of the stop of follow-up as control individual and the start of follow-up as exposed individual will coincide.

Definition of Exposure

The primary analysis considered a minimum duration of exposure to minocycline (42 days) or the alternative treatments equal to the recommended minimum

duration of treatment with these drugs for the treatment of acne – based on the information of the drugs' Summary of Product Characteristics, the British National Formulary (British National Formulary, 2016) or the NICE guidelines (National Institute for Health and Care Excellence, 2016). Duration of treatment was calculated using the recorded information for the total quantity for the prescribed product and the numeric daily dose prescribed for the event. Where this information was missing (8.3% of prescriptions had missing information), the mean obtained from all other prescriptions of the same drug product was imputed. Continuous or discontinuous exposure was calculated by considering the starting date for individual prescriptions and their duration of treatment and accounting for overlapping prescriptions or temporal gaps, respectively (Figure 1). A temporal gap of up to seven days was considered as continuous use.

Identification of Patients with Severe Mental Illness (SMI)

The outcome for this study was the first recording of a diagnosis of SMI identified using the validated Severe Mental Illnesses codes list created by Hardoon et al. (2013), which classifies codes as schizophrenia, bipolar and other psychotic disorders (Supplementary Table 4). Individuals with any recorded diagnosis of SMI before the start of the follow-up period were not included in the study.

Sensitivity Analysis

A sensitivity analysis was conducted where a minimum duration of treatment was not established and therefore, the follow-up period was taken to start at the first prescription of minocycline or alternative antibiotics. Patients in the control cohort were switched to the exposed cohort at the date of receipt of a minocycline prescription (Figure 2).

Both the primary and sensitivity analyses were adjusted for covariables: age, gender, time of exposure to minocycline or alternative drugs, socioeconomic deprivation status, calendar year and exposure to co-medications measured at the start of follow-up. Socioeconomic deprivation status, which has been described to have a potential relationship with the incidence of SMI (Kirkbride et al., 2012), was represented as the practice Index of Multiple Deprivation (IMD) score, the official measure of relative deprivation for small areas in England. Multiple deprivation is articulated as an accumulation of discrete dimensions of deprivation such as financial, health, education, services or crime. In this study the practice IMD, which uses the general practice postcode and data, was provided as quintiles of the deprivation score or rank to prevent disclosure of practice area. Co-medications of interest were those agents potentially related to the incidence of SMI and are listed in Table 1.

In separate analyses, we investigated the potential relationship with 1) gender, 2) exposure to doxycycline, or 3) type of SMI diagnoses, whilst considering a

minimum duration of treatment (as per primary analysis). The potential relationship between gender and incidence of schizophrenia, which has been observed to be higher in young men than women (Castle et al., 2000), was analysed by stratifying the analyses based on gender. To assess the potential relationship between the incidence of SMI and exposure to doxycycline, which is chemically similar to minocycline and can also penetrate the blood-brain barrier (Domercq and Matute, 2004), we conducted a sensitivity analysis with two main treatment options: minocycline and doxycycline (exposed cohort) and tetracycline, lymecycline, oxytetracycline and erythromycin (control cohort). All prescriptions of these drugs were considered as continuous treatment if they overlapped or finished and started on consecutive days, allowing for a treatment gap up to 7 days. Finally, we assessed the relationship between minocycline and schizophrenia specifically, defining the output as the first recorded diagnosis of a schizophrenia-related code only.

Results

The total number of individuals with at least one recorded acne diagnosis at ages 15-20 years old between 1991 and 2005 and at least twelve months of recorded CPRD data was 61 744, of whom 25 288 were female (41%) and 36 456 male (59%). 19 903 individuals (32%) had a recorded prescription of minocycline (6392) and or alternative oral antibiotic (16 057) before the first diagnosis of acne

after at least 12 months of recorded follow-up on the CPRD and were not included in the study. From the remaining 41 841 individuals, 4930 (11.8%) were prescribed minocycline but never an alternative treatment; 27 484 (65.7%) individuals were prescribed an alternative treatment but never minocycline; and 9427 (22.5%) individuals were prescribed both.

[Insert Figure 3]

Figure 3. Construction of the study cohorts in the primary and sensitivity analyses.

After the application of inclusion and exclusion criteria in the primary analysis (Figure 3), the final number of individuals in the control and exposed cohorts were 13 248 and 14 393, respectively. The distributions of men and women were 36.6% females and 63.4% males in the control cohort, and 34.6% females and 65.4% males in the exposed cohort.

The average follow-up time in the control cohort (6.53 years) and in the exposed cohort (8.95 years) was statistically significantly different (t test, $p<0.001$). 46.64% of all patients in the minocycline-exposed group (n = 6180) and 38.93% (n = 5604) of those prescribed an alternative treatment for acne were followed up from the start date until the final data collection date. 45.75% percent of all study participants (6849 individuals exposed to minocycline and 5798 individuals in the control cohort) were followed up until they transferred to another primary

care practice, while 0.23% of individuals were followed up until their recorded death date. Follow-up was stopped for 2816 (19.57%) individuals in the control cohort when prescribed minocycline, while 108 (0.82%) and 84 (0.58%) in the exposed and control cohorts, respectively, were censored when prescribed isotretinoin. Characteristics of the individuals in the two cohorts and their standardised differences (Austin, 2009) are presented in Table 1, while the median duration of exposure to minocycline or alternative treatment in the primary analysis are shown in Table 2.

Table 1. Characteristics of the individuals in the exposed and control cohorts presented as number of individuals (n) and percentages. (IMD: Practice Index of Multiple Deprivation being IMD1 = least deprived and IMD5 = more deprived). Standardised differences greater than 0.1 (*) are considered meaningful.

Characteristics	Minocycline-exposed individuals (n=13 248)	Alternative treatment-exposed individuals (n=14 393)	Standardised differences
Median age at start-date	17.42 ± 5.17	17.61 ± 7.99	0.074
Sex, n (%)			
Male	8405 (63.4)	9418 (65.4)	0.042
Female	4843 (36.6)	4975 (34.6)	0.042
Calendar year at start date, n (%)			
1987-1991	573 (4.33)	533 (3.7)	0.032
1992-1996	3010 (22.72)	2122 (14.74)	0.206*
1997-2001	3581 (27.03)	3774 (26.22)	0.018
2002-2006	5455 (41.18)	6353 (44.14)	0.060
2007-2011	592 (4.47)	1286 (8.93)	0.179*
2012-2015	37 (0.23)	325 (2.26)	0.184*
Deprivation status, n (%)			
IMD1	2534 (19.1)	3013 (20.9)	0.045
IMD2	2554 (19.3)	2802 (19.5)	0.005
IMD3	2350 (17.3)	2809 (19.5)	0.057

IMD4	2721 (20.5)	2729 (19.0)	0.038
IMD5	3089 (23.3)	3040 (21.1)	0.053
Exposure to co-medications, n (%)			
aspirin	172 (1.3)	167 (1.16)	0.013
celecoxib	8 (0.06)	11 (0.08)	0.008
methotrexate	5 (0.04)	1 (0.01)	0.019
methylphenidate	32 (0.24)	35 (0.24)	0.000
metoclopramide	383 (2.89)	416 (2.89)	0.000
warfarin	7 (0.05)	5 (0.04)	0.005
hormonal treatment	1,484 (11.21)	1,744 (12.12)	0.028
antidepressants, hypnotics and sedatives	247 (1.86)	292 (2.03)	0.012

In the primary analysis, the crude incidence rate (IR) of SMI in the minocycline-exposed cohort was 6.65 per 10,000 person-years (95% confidence interval [CI] 5.33-8.28) and 6.39 per 10,000 person-years (95% CI 4.96-8.22) in the alternative treatment cohort. The crude IRR showed no evidence of a relationship between the use of minocycline and the incidence of SMI (IRR 1.04, 95% CI 0.74-1.46, p=0.816), which was consistent with the results from the analysis adjusted for covariables (IRR 0.96, 95% CI 0.68-1.36, see Table 3).

Table 2. Median duration of treatment in days and interquartile range (IQR) with any treatment (overall) and depicted by minocycline or alternative acne treatments in the exposed and control cohorts in the primary analysis.

	Treatment	Continuous treatment[a] *Median (IQR)*	Total duration of exposure[b] *Median (IQR)*
Exposed cohort	**Overall**	**56 (50)**	**279 (442)**
	Minocycline	56 (55)	336 (469)
	Doxycycline	29 (54)	168 (321)
	Lymecycline	56 (44)	202 (322)
	Oxytetracycline	45 (32)	170 (273)
	Tetracycline	32.5 (28)	168 (342)

	Erythromycin	28 (49)	164 (280)
Control cohort	**Overall**	**56 (62)**	**376 (476)**
	Minocycline	-	-
	Doxycycline	56 (64)	312 (477)
	Lymecycline	58 (55)	403 (495)
	Oxytetracycline	60 (64)	396 (478)
	Tetracycline	56 (70)	358 (400)
	Erythromycin	50 (46)	222 (295)

[a] Days exposed to the drug as defined by one recorded prescription.
[b] The sum of different exposure periods recorded as different but overlapping recorded prescriptions of the same active ingredient.

In the sensitivity analysis, the final number of individuals in the control and exposed cohorts was 32 219 (61.3% male) and 14 071 (63.1% male), respectively (Figure 3). The mean duration of follow-up was 6.7 years in the control cohort and 9.1 years in the exposed cohort. The IR of SMI in the minocycline-exposed cohort was 6.9 per 10,000 person-years (95% CI 5.61-8.52) and 6.31 per 10,000 person-year (95% CI 5.35-7.43) in the alternative treatment cohort. As shown in Table 3, the IRR showed no evidence of an association between the use of minocycline and the incidence of SMI, even when adjusting for all covariables (IRR 1.08, 95% CI 0.83-1.42, p=0.566).

Gender analyses showed no relationship between treatment with minocycline and incidence of SMI either for males or females when adjusted for covariables, in the same way that the analysis considering recorded diagnoses of schizophrenia-related codes only did not show evidence of a relationship between exposure to minocycline and schizophrenia (Table 3). Similar results were

obtained when recorded prescriptions of minocycline and doxycycline were considered as the drugs of interest (Table 3).

Detailed results for all the adjusted analyses are shown in Supplementary Table $8_{a\text{-}f}$. A relationship between gender (as a covariable) and incidence of SMI was observed in the primary, sensitivity and doxycycline-minocycline analyses (Supplementary Table $6_{a,b,d}$). A higher incidence of SMI was related to individuals exposed to acne treatment (in both the exposed and control cohorts) for up to one year in the sensitivity and doxycycline-minocycline analyses (Supplementary Table $6_{b,e}$). A relationship with socioeconomic deprivation status was observed only when considering exposed individuals to those prescribed doxycycline and/or minocycline (Supplementary Table 6_{e}).

Table3. Incidence Rate Ratio (IRR) of severe mental illness (SMI) in the different analyses adjusted for covariables (age, gender, time of exposure, calendar year, socioeconomic deprivation status and co-medications).

	Person Years	SMI Cases	Adjusted IRR for covariables (95% CI)
Primary analysis (minimum duration of treatment)			
Alternative treatment	93 961	60	1
Minocycline	118 891	79	0.96 (0.68-1.36) p=0.821
Sensitivity analysis (no minimum duration of treatment)			
Alternative treatment	225 164	142	1
Minocycline	127 332	88	1.08 (0.83-1.42) p=0.566
Gender analysis: Males			

Alternative treatment	64 624	48	1
Minocycline	78 527	60	0.94 (0.63-1.38) p=0.738
Gender analysis: Females			
Alternative treatment	29 291	12	1
Minocycline	40 364	19	1.10 (0.52-2.32) p=0.807
Doxycycline and/or minocycline			
Alternative treatment*	131 838	79	1
Doxycycline/minocycline	145 059	96	1.15 (0.85-1.56) p=0.364
Diagnosis: Schizophrenia			
Alternative treatment	94 202	14	1
Minocycline	119 217	35	1.67 (0.89-3.15) p=0.111

*Alternative treatments: tetracycline (oral), lymecycline (oral), oxytetracycline (oral), erythromycin (oral).

Discussion

Main Findings

Our study shows no evidence to support a preventative effect of exposure to minocycline during adolescence on the incidence of SMI in humans. These conclusions were consistent irrespective of considering a minimum duration of treatment and did not differ when ruling out the effect of the similar tetracycline doxycycline or when considering only schizophrenia-related diagnoses.

To the best of our knowledge, this is the only study that retrospectively assessed the relationship between exposure to minocycline and the incidence of SMI in a large population and over a long study period. Minocycline was for a long time the first line treatment for moderate and severe acne and, therefore, acne

diagnosed patients represent an appropriate population to assess the effects of long-term exposure to minocycline while allowing for the identification of a control group as those patients prescribed an alternative oral antibiotic, which include oral tetracyclines (tetracycline, oxytetracycline, doxycycline, and lymecycline) and oral erythromycin. Age 15-20 years is the more likely age for acne diagnosis and treatment (Zaenglein et al., 2016) whilst the onset of SMI occurs usually later in early adulthood. Hence, these individuals were selected in this study to focus on the potential effect of minocycline in adolescence and to reduce confounding by treatment with other substances.

The comparison of baseline covariables using absolute standardised mean differences shows the similarity of the two cohorts. Although there is no universally accepted threshold to indicate the presence of meaningful imbalance (Austin, 2009), only three groups of the stratification of calendar year showed a value higher than the typically accepted 0.1 threshold (although they remained ≤ 0.2). These differences might reflect a change in the prescribers' preferences towards minocycline or alternative treatments, but the adjusted analyses showed no impact in the conclusion of this study (Supplementary Table 8). Results were adjusted for all mentioned covariables: age, gender, socioeconomic deprivation status, time of exposure to minocycline or the alternative treatment, calendar year and co-medications described to have a potential relationship with SMI.

Although there is not conclusive evidence of the link between exposure and SMI, we adjusted for co-medications described to have a potentially beneficial effect on SMI similar to minocycline: warfarin (Hoirisch-Clapauch and Nardi, 2013), estrogens in the form of combined oral contraceptives (Bump et al., 2013), methotrexate (Chaudhry et al., 2015) and the NSAIDs celecoxib (Akhondzadeh et al., 2007) and aspirin (Laan et al., 2010). Those drugs described to potentially worsen the symptoms of SMI were also considered: isotretinoin (Ludot et al., 2015), methylphenidate (Shyu et al., 2015) and metoclopramide (Lu et al., 2002). We also adjusted for medications that might be indicative of current psychiatric problems – such as prescription of antidepressants, hypnotics or sedatives. Regarding gender, the distribution of men and women were similar in the two cohorts, with a larger number of males in both groups, which might be explained by the selection of alternatives to oral antibiotics, such as hormonal treatments as a first line treatment of acne in young women, or the wish to avoid systemic treatments in females of childbearing age.

Patients in the minocycline exposed group had a slightly longer average follow-up time. We believe this difference in follow-up time occurred because it was possible for control group patients to move to the minocycline control group. Minocycline exposed group patients remained in their assigned cohort arm,

irrespective of any change in treatment for acne, as no protective effect of non-minocycline antibiotic treatment was expected.

Although other tetracyclines, especially doxycycline, have been reported to share the anti-inflammatory and neuroprotective properties of minocycline, they have not shown a similar efficacy in the same models (Garrido-Mesa et al., 2013) probably because of minocycline's multiple targets, higher lipophilicity and capacity to cross the blood-brain barrier (BBB) compare to other tetracyclines. Erythromycin, a macrolide antibiotic, does not cross the BBB well and its concentrations in the cerebrospinal fluid are low (Sweetman, 2016). To the best of our knowledge, only minocycline has been investigated as a protective or adjuvant therapy for the treatment of schizophrenia. It is not expected, therefore, that exposure to other tetracyclines or erythromycin have a relationship with the incidence of SMI. To assess this in detail, however, we conducted an analysis where minocycline and the only tetracycline that crosses the BBB in a similar – although smaller – extent, doxycycline, were considered the drugs of interest. Results were consistent with those in the primary and sensitivity analyses.

The observed relationship between gender and incidence of SMI is in keeping with the established literature which shows a higher incidence of SMI in young men compared with women (Castle et al., 2000), possibly due to a protective effect of estrogens on the emergence of SMI. Although this conclusion has not

been reached in some studies (Pedersen et al., 2014), we allowed for this potential beneficial effect and exposure to estrogens prescribed before the start of the follow-up period was considered a potential covariable. Exposure to this or other mentioned co-medications did not have a great impact on the results, possibly because of the low probability of adolescent individuals being exposed to these drugs. In the case of exposure to methylphenidate – commonly used to treat attention-deficit disorders (ADD) in children – the results showed a higher incidence of SMI in the sensitivity and doxycycline-minocycline analyses, while a relationship between exposure to antidepressants and later incidence of SMI was found in all our studies except for the sub-analysis for women. Although the studies described here were not designed for this purpose and give results that differ considerably between the different types of analyses (Supplementary Table 7), our findings do suggest that individuals diagnosed with ADD may be diagnosed more often with SMI and that individuals with recorded prescriptions of methylphenidate or antidepressants during adolescence may have a higher incidence of SMI.

Evidence from Animal Studies

Adolescence is a relevant period of brain development characterized by dramatic changes in brain growth and connectivity through the creation of efficient neural pathways through synaptic refinement and it is, therefore, a period of

susceptibility to developmental disturbances induced by endogenous and exogenous factors (Bossong and Niesink, 2010), especially in neurodevelopmentally vulnerable subjects with genetic predisposition or prenatal infectious histories (Giovanoli et al., 2013). Mouse models showed that offspring exposed to combined prenatal immune challenge and peri-pubertal stress showed signs of central nervous system inflammation in the form of microglial overactivation (Giovanoli et al., 2016). Microglia are activated in response to brain injuries and an extensive list of immunological and pro-inflammatory stimuli, leading to excessive and inappropriate release of toxic factors and pro-inflammatory cytokines (Takahashi et al., 2016). The relationship between psychiatric disorders and microglial activation in humans has been suggested by post-mortem brain and positron emission tomography (PET)-based studies (Takahashi et al., 2016). Exposure to anti-inflammatory and microglia-activation inhibitory agents such as minocycline during adolescence has thus been proposed to have a potential preventative effect on the onset of these disorders and in the treatment of symptoms in early schizophrenia (Chaudhry et al., 2012; Giovanoli et al., 2016).

Prior evidence for this preventative effect of minocycline comes, however, from a small number of animal studies and is not readily extrapolated to the clinical setting. Most of these studies used pharmacological models of schizophrenia

induced by dizocilpine maleate (Levkovitz et al., 2007; Zhang et al., 2007) or ketamine (Monte et al., 2013), which affect specific systems altered in schizophrenia (glutaminergic and dopaminergic system) and lead to models with cognitive deficits that resemble those seen in the disorder (Jones et al., 2011). Exposure to minocycline in these models is arranged close to time of administration of the inductive agents and so these experiments are of limited value in assessment of the long-term preventative effects of the drug.

Schizophrenia and other SMI are multifactorial neurodevelopmental disorders influenced by both genetic and environmental factors that cannot be replicated by a pharmacological model only. Giovanoli et al. (2016) assessed the preventative potential of minocycline treatment in an environmental two-hit mouse model of schizophrenia combining prenatal immune activation and peri-pubertal stress in mice, with the oral pre-symptomatic administration of minocycline taking place 24 hours before and during exposure to stressors. Although this approach provides a more realistic model and can be a useful tool to advance our understanding of the etiological basis of the disease, it still presents limitations for the evaluation of the long-term effect of minocycline in the clinical setting.

Strength and Limitations of the Study

Conducting a randomized clinical trial to assess the preventative effect of long-term exposure to minocycline during adolescence on the incidence of SMI would lead to ethical and economic issues that would make it impracticable. The best approach to address this clinically relevant question is, therefore, the hypothesis-driven study described in this paper (Suissa and Garbe, 2007). The study has been conducted as a prospective observation study were patients were switched from the control to the exposed cohort when they completed a minimum duration treatment with minocycline after a minimum duration treatment with an alternative drug. To avoid potential bias caused by patients contributing follow-up time to both the control group and minocycline-exposed, we conducted a post-hoc analysis using robust standard errors to account for patient clustering. Results remained similar and evidence of a protective effect of minocycline in SMI was not found.

We acknowledge that some patients may have received minocycline or alternative treatment for acne before receiving a diagnosis for acne and these patients were excluded from our cohort analysis. We decided to include patients exposed to minocycline after a diagnosis for acne to increase comparability between the exposed and non-exposed group.

The main limitations of this study are those related to use of electronic primary care data. Recorded prescriptions in the CPRD data do not guarantee patients'

adherence to the treatment and exposure to the drug. We should also acknowledge that treatment with minocycline could be prescribed through routes not recorded in the CPRD, such as secondary care units. The large population and length of the study period, however, are likely to mitigate against systematic bias. The mean duration of continuous exposure to minocycline was 336 days (11 months) and we should recognize the possibility that the preventative effect might only be seen after longer exposure periods. Several potential confounders were identified and we adjusted for these covariables in our final model. The comparison of baseline characteristics showed the similarity of the two cohorts and, therefore, a propensity score matching approach was not considered pertinent. Apart from the mentioned demographic and treatment covariables other aspects, such as somatic co-morbidities or parental mental disorders, should be considered in further studies which do not rely only on primary care data. Although this study assessed the preventative effect of minocycline before the onset of the disease, the beneficial effect of the add-on therapy of minocycline could not be evaluated. More research should be conducted, therefore, to provide insights on the role and biological basis of minocycline as an adjuvant therapy for SMI. Nevertheless, the findings described here represent a crucial contribution to the field, and the robustness of the study design, the large survey population, and the long study period ensure their reliability. The conclusions of this study

should be considered when conducting future research and especially when planning clinical interventions.

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Contributors

FG and DT developed the idea for the study design to address the research question. MHZ conducted the literature review. MHZ and DJB conducted the first study design and processing of raw data with further inputs from FG, LMH, RB, and DT. RB introduced MHZ to the STATA syntax and provided guidance on data preparation and analysis. MHZ wrote the syntax with the input of RB. MHZ wrote the draft of this report, and DJB, FG, DT, LMH, and RB contributed comments and suggestions. MHZ and DJB revised the final version.

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