Abstract

Allopurinol, a first-line gout treatment drug in Australia, was assessed as a wastewater-based epidemiology biomarker of gout via quantification of the urinary metabolite, oxypurinol in wastewater. The in-sewer stability of oxypurinol was examined using laboratory-scale sewer reactors. Wastewater from 75 wastewater treatment plants across Australia, covering approximately 52% (12.2 million) of the country's population, was collected on the 2016 census day. Oxypurinol was quantified in the wastewater samples and population-weighted mass loads calculated. Pearson and spearman rank-order correlations were applied to investigate any link between allopurinol, other selected wastewater biomarkers, and socio-economic indicators. Oxypurinol was shown to be stable in sewer conditions and suitable as a WBE biomarker. Oxypurinol was detected in all wastewater samples. The estimated consumption of allopurinol ranged from 1.9 to 32 g/day/1000 people equating to 4.8 to 80 DDD/day/1000 people. The prevalence of gout across all tested sewer catchments was between 0.5% to 8%, with a median of 2.9% nationally. No significant positive correlation was observed between allopurinol consumption and alcohol consumption, mean age of catchment population, remoteness or higher socioeconomic status. There was a significant positive correlation with selective analgesic drug use. Wastewater analysis can be used to study gout prevalence and can provide additional insights on population level risk factors when triangulated with other biomarkers.

Keywords: *allopurinol, oxypurinol, gout, rheumatic disease, wastewater-based epidemiology*

1. Introduction

Chronic musculoskeletal and joint disorders lead to disability as reported by the global burden of disease study [1, 2]. One of the core groups of chronic musculoskeletal and joint disorders is arthritis, a broad term covering a number of inflammatory arthritic conditions, such as gout [3]. Gout is an acute form of metabolic inflammatory arthritis, triggered by uric acid deposition in and around peripheral joint tissues. Constantly high levels of uric acid lead to the disposition of monosodium urate crystals in the peripheral joint areas and causes the painful symptoms of gout [4]. Chronic deposition of monosodium urate crystals can lead to joint damage, metabolic syndrome, renal diseases, and cardiovascular diseases. Gout is listed as a cardiovascular risk factor that leads to reduced work productivity, and affects the quality of life and longevity [3, 5].

Gout prevalence in developed countries is on the increase and is a serious health problem among the elderly [3]. A study in the USA revealed that roughly 3.4% of the adult population suffered from gout between 2007 and 2008 [6]. Gout is thought to affect almost 3% of Canadian adults [7], around 2.5% of residents of the UK and the Netherlands [8, 9]. However, France and Italy have only a gout prevalence of around 1% [10, 11]. The prevalence of gout in Australia has been reported to be between 1.5 and 9.1 % based on the patient and survey data [12-15]. In contrast, a self-report survey, concluded that only 0.8% of Australians suffered from gout [16]. However, previous Australian gout studies have surveyed only specific or small parts of the entire Australian population and provide a low spatial coverage of prevalence.

Age, sex and socio-economic factors [17-20]; constant hyperuricemia [21-24]; genetics [25-27]; current diseases, medications and obesity [28-34]; food, diet, alcohol consumption [35-43], and exposure to lead [44-46] are all considered as gout risk factors. Healthy lifestyle patterns and a change in diet are key elements of gout management. However, the advantage of these is not well understood [47, 48] and even without comprehensive dietary education, it is difficult to manage or reduce gout risk factors. Urate lowering drugs are more efficient than diet or lifestyle management. Allopurinol (xanthine oxidase inhibitor) is recommended as a first-line medicine for gout patients in Australia [49] as it is cheaper than other options, leading to almost seven times more doses being sold per year when compared to feboxostat (per year report from Pharmaceutical Benefits Schedule Item Reports) [50, 51]. Allopurinol is extensively

metabolized into oxypurinol (61-69%), allopurinol-1-riboside (10%) and two riboside conjugates of oxypurinol (1-3%) and excreted in urine [52].

The Pharmaceutical Benefits Scheme (PBS) in Australia provides statewide sales data but has too low a resolution to capture real-time drug consumption data and spatial differences, especially in city or rural-urban areas. In contrast, wastewater-based epidemiology (WBE) has developed into a complementary approach that can provide near real-time drug monitoring at a population level [53, 54]. Wastewater is a mixture of sewage from a community serviced by a wastewater treatment plant, and hence can be considered a source of pooled urine that can reveal a wealth of information on the sewer catchment inhabitants [55]. It is based on the quantitative analysis of biomarkers in the influent of wastewater treatment plants (WWTPs) [56-59]. The ability of WBE to cover large populations, and determine patterns of drug consumption over time, has led to its application as a tool for measuring nicotine consumption [60-64], alcohol use [60, 63, 65, 66], illicit drug consumption [67-72] and for public health monitoring [73-75] in different countries. Recent WBE studies have demonstrated correlation between consumption of some drugs and socioeconomic indicators of the respective populations [76-78]. A suitable WBE biomarker must have specific characteristics such as i) excreted mainly via urine in consistent amounts and must have adequate concentrations for instrumental measurement once diluted in wastewater ii) unique to human metabolism and iii) sufficiently stable in wastewater [79]. From clinical perspectives, oxypurinol meets these criteria [80] but its stability in sewers has yet to be assessed.

The Australian census data delivers socio-economic measures to calculate different socio-economic indexes for areas (SEIFA) as well as descriptors (income, house, employment, education and so forth) every 5 years. Based on the SEIFA descriptors, a broader indicator named indices of relative socio-economic advantage and disadvantage (IRSAD) which summarises information about the economic and social conditions of people and households within an area. This includes an index which captures the relative advantage and disadvantage on inhabitants, and has been used in different research fields [81-83]. Robinson et al, 2015 [12] previously correlated the socioeconomic status of the Australian population with gout prevalence by calculating patient survey data over a five year period. However, this study covered only 3% of the Australian population, which may not be truly representative of Australia as a whole. Recently Choi et al., 2019 [84] demonstrated that socio-economic data

from the Australian 2016 census could be used in combination with WBE to identify relationships between catchment specific socio-demographic parameters and WBE biomarkers to reveal the population behaviour and health status.

Due to the lack of suitable methods that can cover the largest population, and in the absence of appropriate Australian prevalence data, this study aimed to estimate gout prevalence in Australia by wastewater analysis with sub-aims of (i) testing the suitability of oxypurinol as a WBE biomarker (ii) comparing population-weighted mass loads with gout disease risk factors and other WBE biomarkers as well as (iii) assessing SEIFA indicators that may be potential risk factors. To date, this is the first WBE study to evaluate gout prevalence.

2. Materials and Methods

2.1 Sample Collection and Processing

Wastewater influent samples (24 hr composite) from 75 wastewater treatment plants (WWTPs) across Australia were collected to coincide with the 2016 Australian census (9th August 2016). The WWTP catchments were in the states of New South Wales (NSW), Queensland (QLD), South Australia (SA), Victoria (VIC), the territories of the Australian Capital Territory (ACT), and the Northern Territory (NT). As per the Australian Bureau of Statistics definition of Remoteness Areas in Australia [85], 28 WWTPs were in major cities; 26 in inner regional areas; 17 in outer regional cities and 4 in remote areas. Census day was selected as the sampling period as samples can be matched the accurate catchment population, demographic data and socio-economic variables from the census as outlined by O'Brien et al., 2018 and Tscharke et al., 2019 [86, 87]. This study covered approximately 52% of the Australian population (12.2 million people).

Sample collection was conducted as previously described by O'Brien et al., 2019 [54]. Each wastewater sample was defrosted, filtered with regenerated cellulose syringe filters (0.2 µm) and aliquoted into 1 mL amber vials. Then 750 µL was drawn and mixed with 250 µL MiliQ water and spiked with 10 ppb (µg/L) oxypurinol internal standard. Samples were then analysed via direct injection with an LC-MS/MS system.

2.2 LC-MS/MS Parameters

A direct-injection LC-MS/MS method based on Funke et al., 2015 [88] method was developed for the determination of oxypurinol, the main urinary metabolite of gout disease treatment drug allopurinol in wastewater. Optimal declustering potential (DP), collision energy (CE) and collision exit potential (CXP) for fragments of each analytical standard (SI, Table S1) were determined by infusion into a Sciex 6500+ Triple Quad mass spectrometer (Ontario, Canada). Chromatographic separation was achieved with a Synergi 4µm Hydro RP 80 Å LC 150 x 3 column. Mobile phase A was MiliQ water and 0.1% formic acid. Mobile phase B was methanol and 0.1% formic acid. The gradient began at 5% and was held at 5% to 2 minutes, followed by a linear increase to 70%B at 8 minutes, held at 70% until 12 minutes, decreased to 5% at 12.5 minutes, and held at 5% until the end of the run at 18 minutes.The flow rate was set to at 0.45mL/min. A six-point calibration curve ranging from 5-200 µg/L was made in MilliQ water for quantification. The method was validated for limit of detection (LOD), limit of quantitation (LOQ), accuracy, precision, linearity (SI, Table S2) following by The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines [89]. Multiquant™ (AB Sciex, version 3.0) software was used for all data quantification.

2.3 Calculations

2.3.1 Social, Demographic and Economic, Disease Prevalence and Primary Health Networks (PHNs) Data

The Index of Relative Socio-economic Advantage and Disadvantage (IRSAD), developed by the Australian Bureau of Statistics (ABS), uses individual SEIFA indicators to depict the economic and social conditions of people and households, aggregated by area (SI, Table S3). It indicates the relative social advantage and disadvantages with respect to their positive and negative scores. For each catchment, IRSAD was calculated using Statistical Level 1 (SA 1) geographic resolution of the Australian census, as well as the individual SEIFA descriptors. Detailed procedures have already been described [90].

The obesity, overweight, and diabetes prevalence data was reported by Australia's Health Tracker by Area, 2017 [91] for every Local Government Area (LGA) geography as published by the Australian Bureau of Statistics (ABS). To estimate the obesity rates in each wastewater treatment plant catchment, the obesity/diabetes data was aggregated based on the following

cases, and matched to the wastewater catchment boundary: i) where the wastewater catchment area was located fully within the LGA: the obesity/diabetes rate was assumed to be consistent with the catchment area, and ii) when several LGA were contained within one wastewater catchment area: the population-weighted average obesity rate was calculated using the total LGA population and the obesity rate for each LGA contained within the catchment. Primary Health Networks (PHNs) areas were developed by the Department of Health to provide better visual aid of health conditions and the effectiveness of medical services in the whole geographic area. Primary Health Networks (PHNs) gout prevalence was estimated via the aggregation (population-weighted average) of wastewater catchments within each area. Wastewater catchments that were split between PHNs were also aggregated on the proportional area within the PHN [92].

2.3.2 Wastewater Calculations

De facto populations were calculated following previously published methods [87]. Briefly, to estimate WWTP catchment populations, WWTP catchment maps were intersected with geo-referenced de facto population counts using the highest resolution of the 2016 Australian census in GIS software (QGIS version 2.18)[87]. Mass loads were calculated using daily flow rates supplied by the WWTP operators, and the calculated concentrations, calculated as:

$$Mass\ load\left(\frac{g}{day}\right) = Concentration\ \left(\frac{\mu g}{L}\right) X\ Flow\ rate\ \left(\frac{L}{day}\right) \text{.....(a)}$$

Here, concentration in µg/L as measured from LC-MS/MS, flow rate provided by the wastewater treatment authorities in megalitres per day (ML).

$$Daily\ consumption\ \left(\frac{\frac{mg}{day}}{1000}\right) = \left(Mass\ load\ \frac{\frac{g}{day}}{Population}\right) X\ 1000 \text{ .....(b)}$$

In this study, 0.4 g was used as the defined daily dose of allopurinol [93] and excretion factor (0.65) was used from the clinical study reported elsewhere [80]. To convert mass loads to consumed defined daily doses, a back-calculation factor was applied and calculated as follows:

$$Back\ calculation\ factor = \frac{\text{Molecular weight ratio of parent drug and metabolite}}{\text{Excretion factor}} \text{ ..... (c)}$$

By considering 1 allopurinol defined daily dose = 1 patient, allopurinol consumed mg/day/1000 people was converted to Defined Daily Dose (DDD)/day/1000 people, also used as an estimate of gout prevalence reported elsewhere [94].

2.3.3 Correlation Data and Statistical Analyses

Population normalised allopurinol consumption data were plotted with previously analysed and published WBE data sets for calculating statistical significance through pearson and spearman rank-order correlation (see full list of WBE biomarkers, SI, Table S4) [78, 84, 95, 96]. Correlation coefficient (0.5) was selected for both method as the threshold value for statistical significance. We employed both the parametric Pearson correlation and non-parametric Spearman Rank Order correlation analysis in order to capture both the linear and monotonical correlations between the markers. This combination enabled us to avoid any data pre-treatment processes such as the data normalization. In our previous study, we have demonstrated the adequacy of such a threshold for the statistical significance assessment [78].

2.4 Experimental Set up of Sewer Reactors

A requirement of suitable biomarkers for WBE studies is that biomarkers are sufficiently stable within the sewer [79]. As such, laboratory-scale sewer reactors representing a rising main(RM), gravity sewer (GS) and control reactor (CR), were used to determine the stability of oxypurinol in sewers. Wastewater samples were collected from a residential pumping station in Brisbane, Australia and preserved at 4°C for further use. Typical physicochemical parameters of this wastewater include low sulfide (<3 mgS/L), 7.5 pH, 10–30 mgS/L sulfate, and 180–200 mg/L SCOD and methane (<5 mgCOD/L) which is similar to normal domestic sewage. The RM reactor, GS reactor and CR reactor were operated with identical dimensions (diameter 80 mm, height 150 mm), volume (0.75 L), stirring with a magnetic mixer (250 rpm)[97] (for details information section 2.4 in SI). Each reactor experiment was performed in triplicate. Samples were collected and stored in -80° C. Before instrumental analysis, samples were defrosted, and filtered through 0.2 µm regenerated cellulose filters (Agilent, Mulgrave, Australia) and transferred to 1 ml vials and spiked with the internal standard for instrumental analysis. Acesulfame and paracetamol were also measured in each of the samples with acesulfame as a positive control for a stable compound and paracetamol as a positive control for an unstable compound that degrades under GS and RM conditions, as outlined by O'Brien et al., 2017 [98].

3. Results and Discussion

3.1 Suitability of Oxypurinol as a Wastewater Biomarker

The stability of oxypurinol under different sewage conditions was fitted to linear and first-order regressions to model stability up to 24 h (SI, Table S5). Oxypurinol was shown to be stable under all conditions over the test period and meets stability criteria for being an effective WBE biomarker (SI, Fig S1). The results for acesulfame and paracetamol, which are used as benchmarking chemicals, are consistent with previous findings ($t_{10\ \%}$ of >24 hours and 0.12 hours for acesulfame and paracetamol, respectively) [98] (SI, Fig S2)

3.2 Consumed Allopurinol in Different Locations

The estimated consumption of allopurinol across the 75 sewer catchments ranged between 1.9 and 21 g/day/1000 people (SI, Fig S3). Using a Defined Daily Dose (DDD) of 0.4g, allopurinol consumption, expressed as DDD/day/1000, ranged from 4.7 to 80 DDD/day/1000 people in different locations (SI, Fig S4). Inter states and territories comparisons were followed by Australian Capital Territory (ACT), Victoria (VIC), Northern Territory (NT), Queensland (QLD), South Australia (SA), Tasmania (TAS), and New South Wales (NSW) (Fig 1). Noticeable allopurinol consumption differences were not observed between the different remoteness areas (Fig 1).

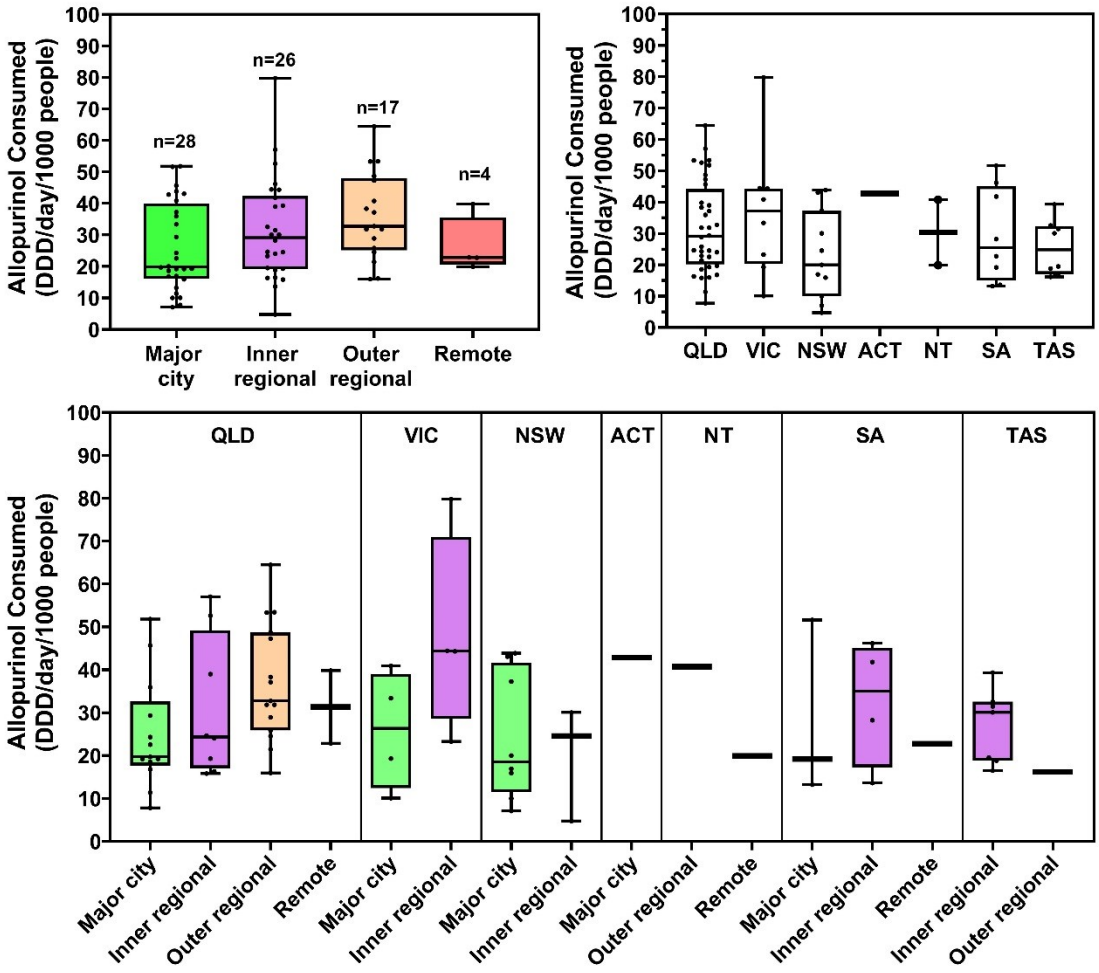

Fig 1: Allopurinol consumed (DDD/day/1000 people) in different Australian territories, states and area of remoteness. [Australian Capital Territory (ACT), Victoria (VIC), Northern Territory (NT), Queensland (QLD), South Australia (SA), Tasmania (TAS), and New South Wales (NSW); box = 25–75 % interquartile range; bar = mean; whisker = min to max data points; one WWTPs in Australian Capital Territory (ACT); one outer regional and one remote WWTPs in NT)]

3.3 Gout Prevalence Estimates Based on Allopurinol

To estimate the prevalence of gout, we assumed one defined daily dose was consumed per patient each day. Based on allopurinol consumption data at population level and conversion of allopurinol (consumed DDD/day/1000) to prevalence (%), gout prevalence estimates ranged from 0.5 % to 8 %, with a median prevalence of 2.9% (SI, Table S6). Gout prevalence by Primary Health Network (PHNs) ranged from 1.2% to 5.5% across Australia (Fig 2, and for more data, SI Table S7). PHN based gout prevalence could contribute monitoring data which could assess health interventions and effectiveness at the population level which medical care is allocated or data collated. To date, this is the first wastewater-based gout prevalence study, hence we are not able to compare with any other wastewater-based gout data. Several studies have previously reported gout prevalence of between 0.8 % to 9.1 % in Australia [16, 99-101]. However, they either reported specific population groups or patient survey data (n= 3005-187,000) for calculating prevalence. Though, our wastewater-based prevalence was similar to the 3-4% prevalence estimates observed in US and Canadian population studies [6, 7].

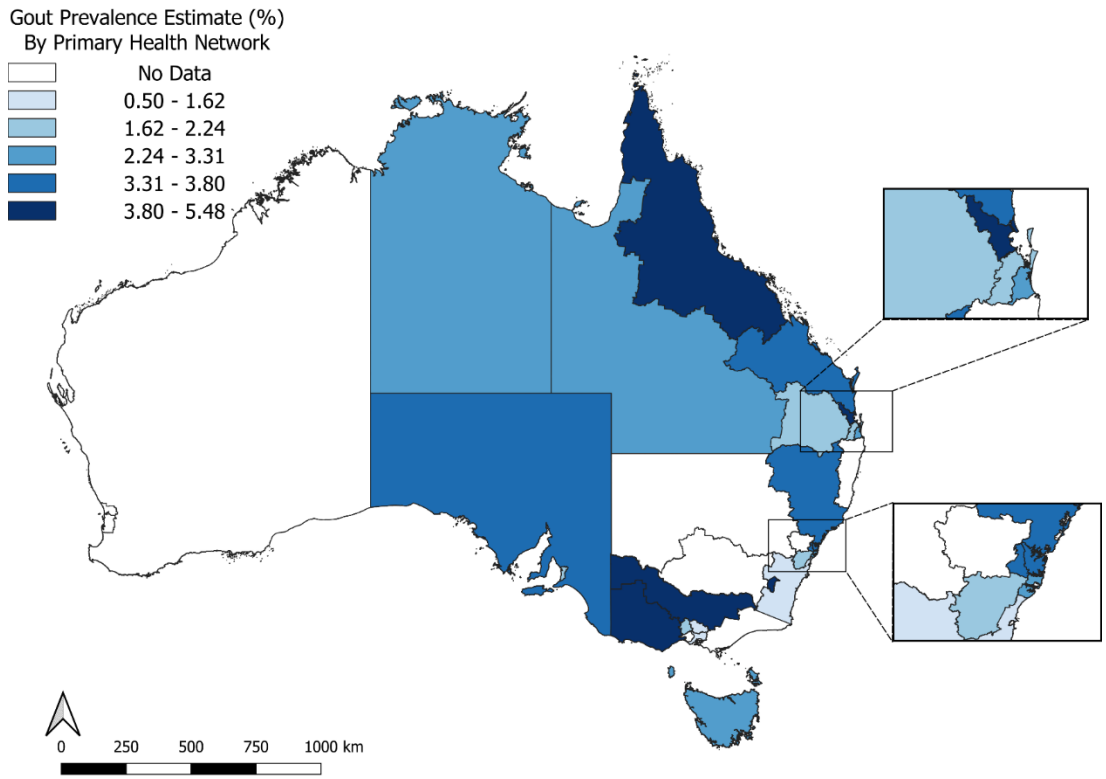

Fig 2: Wastewater-based gout prevalence estimates based on allopurinol consumption using different PHN areas.

3.4 Correlation Study

In this study, a more conservative significance threshold (R=0.5) was used for capturing significant correlations in both pearson and spearman analyses (Fig 3-6).

3.4.1 Correlations Between Gout Biomarker and Age, Alcohol Consumption, and Remoteness of Areas

In our study, there were no significant spearman or pearson correlations with the mean age of the catchment population, nor the level of alcohol consumption at the population level (Fig 3 & 5). It can be hypothesized that population-level gout is not influenced by skews in alcohol and age or prevalence of gout is not high enough in the catchments for that to be reflected in the population-level alcohol or age data. Studies have suggested that residents of rural areas have a lower prevalence of gout than in urban areas [19, 102]. In our study, no significant correlation was observed between remoteness of the catchments and the consumption of allopurinol as has also been reported previously (Fig 3 & 5)[8].

3.4.2 Correlations Between Gout Biomarker and Others Socio-economic Indicators

In our study, there were significant correlations with some socio-economic indicators and gout biomarker. HIGHCAR (% occupied private dwellings with three or more cars) and ATSCHOOL (% people aged 15 years and over who are still attending secondary school users) in catchment population were negatively correlated with gout biomarker in both analyses (Fig 3 & 5). LONE (% occupied private dwellings that are lone person occupied private dwellings) was positively correlated with gout marker.

3.4.3 Correlations Between Gout Biomarker and Other Wastewater Biomarkers

The consumption of naproxen and allopurinol showed a significant positive correlation (Fig 4 & 6). This relationship suggests that since gout can be very painful analgesic drugs might be co-consumed with allopurinol to reduce pain [4]. Similarly, codeine, morphine and pregabalin also showed a positive correlation with allopurinol consumption. An inverse correlation was observed between paraxanthine (a metabolite of caffeine) and allopurinol (Fig 4 & 6). It has also been speculated that noncaffeine xanthines contained in coffee may inhibit xanthine oxidase, thus contributing to lowering serum uric acid levels and less chance of gout [103].

3.5 Limitations

There are a number of limitations that need to be considered when interpreting our study. This study used allopurinol as a proxy for estimating gout prevalence. It is possible that there were undiagnosed gout patients in the sewer catchment who were not being treated with allopurinol. But the percent of undiagnosed gout patients are expected to be negligible as quick medical assistance or treatment is required to reduce severe pain caused by gout. In addition, febuxostat can be prescribed for gout treatment. However, this would only account for 13% of drugs prescribed for gout when comparing to allopurinol sales data reported by the PBS (seven times more allopurinol is prescribed than febuxostat). Moreover, uric acid kidney stones and Familial Mediterranean Fever (FMF) are also treated with allopurinol [12]. Hence, there might be possible sources of allopurinol in sewer catchments not relating to gout disease. Although this study covered 52% of the Australian population, the sampling area does not provide full coverage of Australia as samples were not able to be collected from Western Australia, and so not all Australian states are represented. In addition, we were not also able to compare with certain gout risk factors, such as population-level purine based foods, meat, and seafood consumption or current medications, hypertension and genetic predisposition data which might influence gout onset at the population level in Australia [104, 105]. These data were not available in the literature.

3.6 Conclusion

This study determined the suitability of oxypurinol as a wastewater biomarker and also estimated a median national gout prevalence of 2.9%. The approach also allowed for sewer catchment specific gout prevalence rates to be calculated that ranged between 0.5% to 8%. Correlation studies with socio-economic indicators and WBE biomarkers were also investigated for the drivers of gout disease at the population level. Integrating WBE studies with epidemiological and clinical studies may help provide evidence for the drivers of gout at the population level.

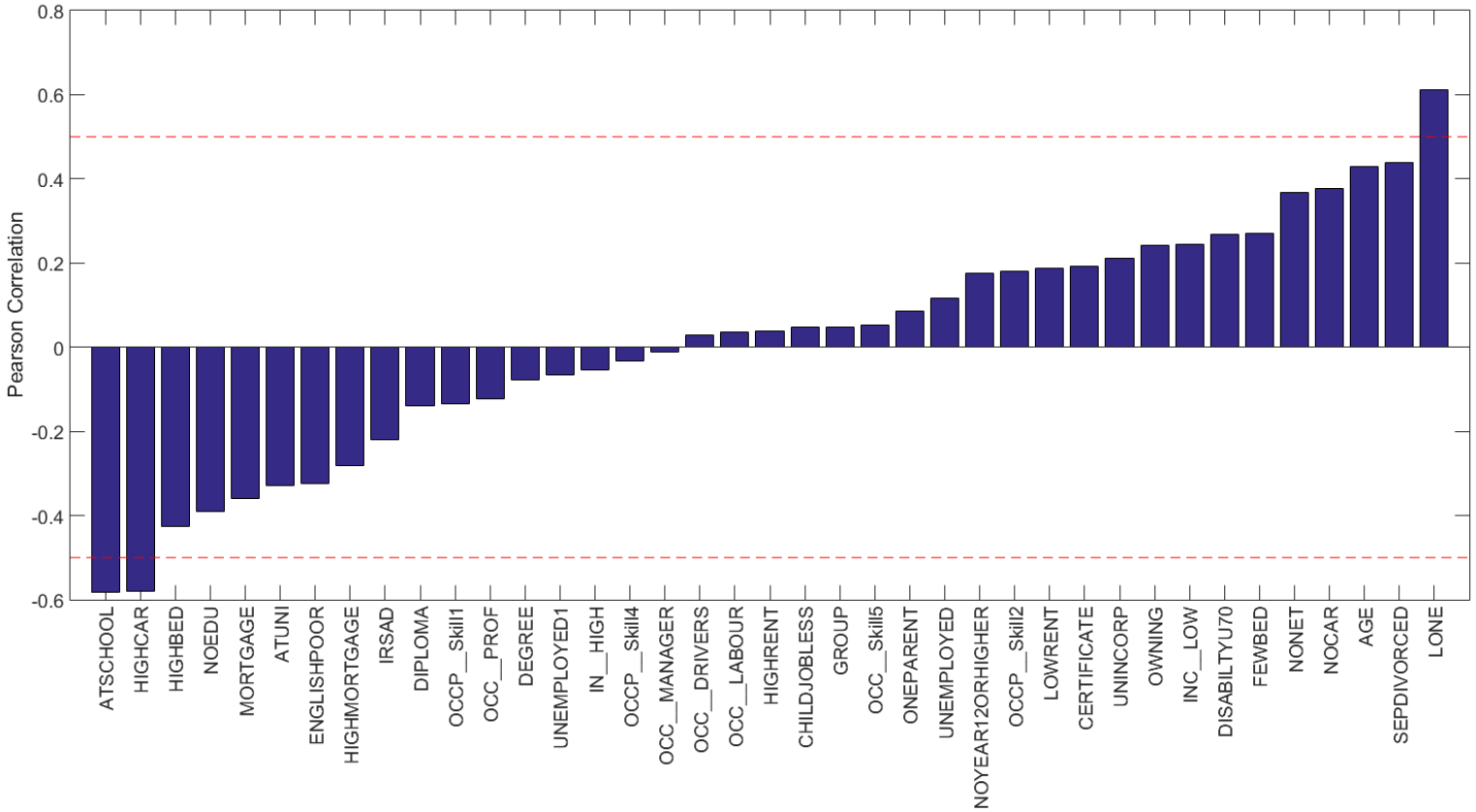

Fig 3: Pearson correlation between allopurinol consumption and socio-economic indicators.

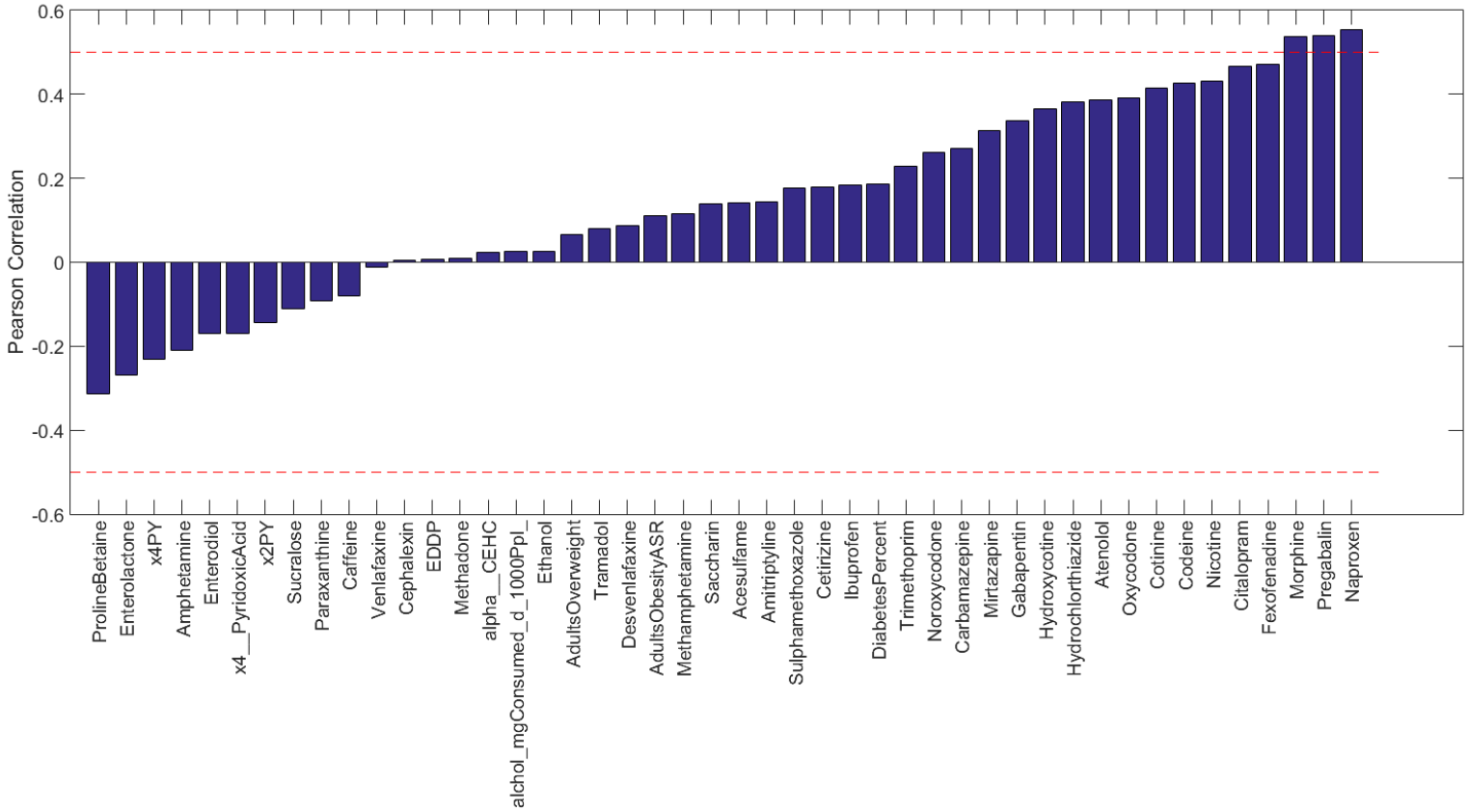

Fig 4: Pearson correlation between allopurinol consumption and other WBE biomarkers.

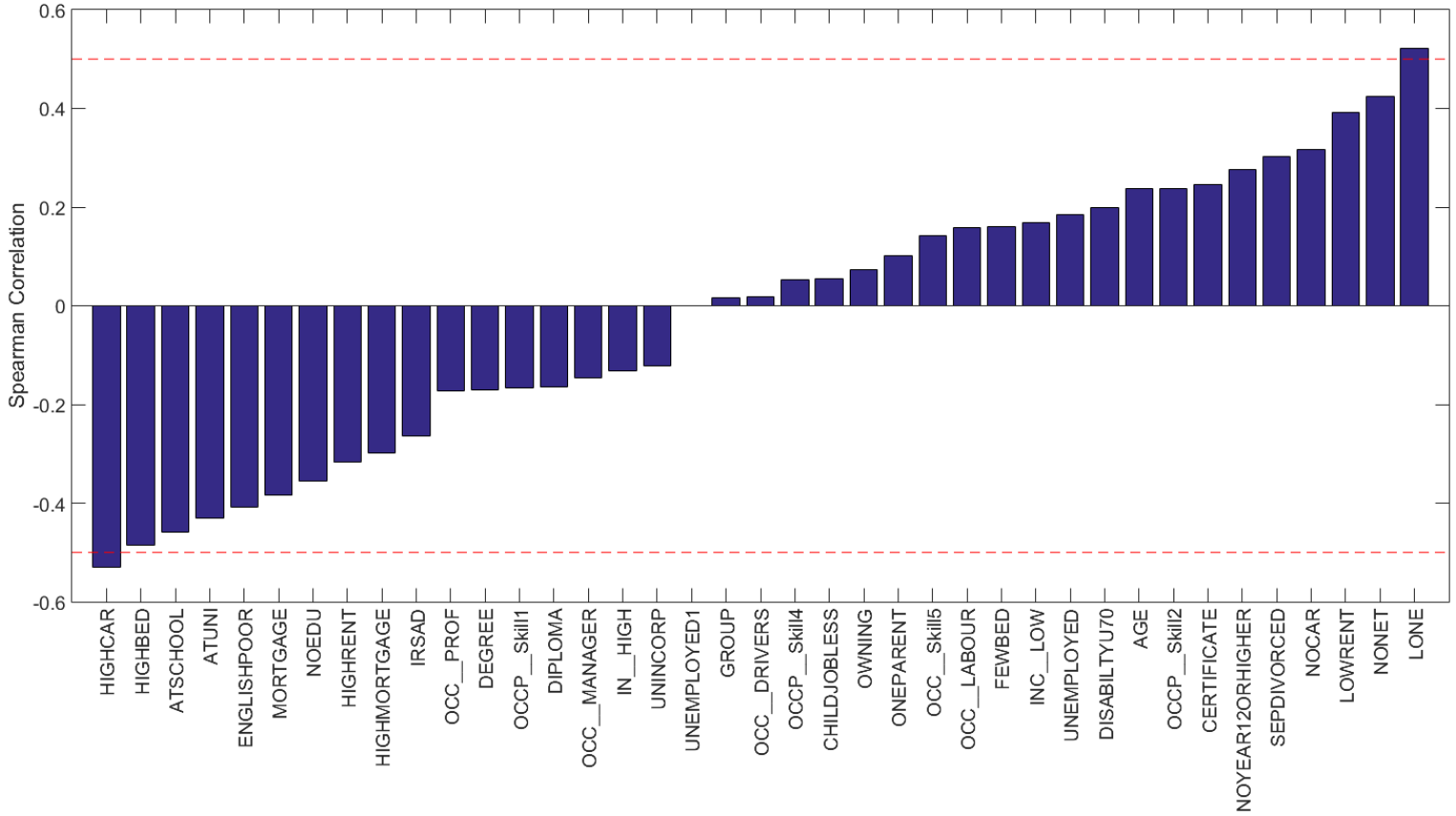

Fig 5: Spearman correlation between allopurinol consumption and socio-economic indicators.

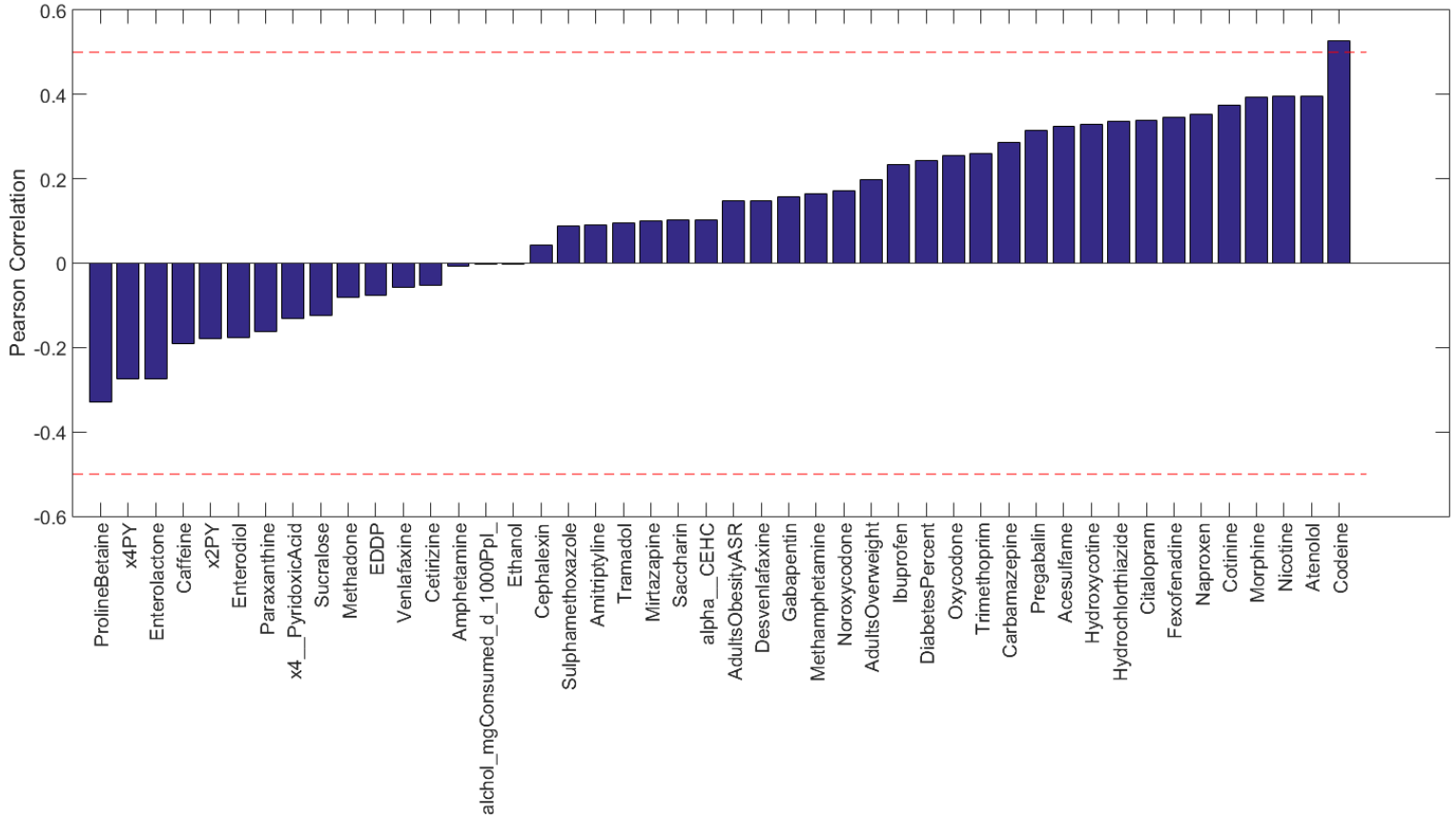

Fig 6: Spearman correlation between allopurinol consumption and other WBE biomarkers.

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