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Cohort profile: early school years follow-up of the Asking Questions about Alcohol in Pregnancy Longitudinal Study in Melbourne, Australia (AQUA at 6)

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ABSTRACT

Purpose The Asking Questions about Alcohol in Pregnancy (AQUA) study, established in 2011, is a prebirth cohort of 1570 mother and child pairs designed to assess the effects of low to moderate prenatal alcohol exposure and sporadic binge drinking on long-term child development. Women attending general antenatal clinics in public hospitals in Melbourne, Australia, were recruited in their first trimester, followed up three times during pregnancy and at 12 and 24 months postpartum. The current follow-up of the 6–8-year-old children aims to strengthen our understanding of the relationship between these levels of prenatal alcohol exposure and neuropsychological functioning, facial dysmorphology, brain structure and function.

Participants Between June 2018 and April 2021, 802 of the 1342 eligible AQUA study families completed a parent-report questionnaire (60%). Restrictions associated with COVID-19 pandemic disrupted recruitment, but early school-age neuropsychological assessments were undertaken with 696 children (52%), and 482 (36%) craniofacial images were collected. A preplanned, exposure-representative subset of 146 children completed a brain MRI. An existing biobank was extended through collection of 427 (32%) child buccal swabs.

Findings to date Over half (59%) of mothers consumed some alcohol during pregnancy, with one in five reporting at least one binge-drinking episode prior to pregnancy recognition. Children's craniofacial shape was examined at 12 months of age, and low to moderate prenatal alcohol exposure was associated with subtle midface changes. At 2 years of age, formal developmental assessments showed no evidence that cognitive, language or motor outcome was associated with any of exposure level.

Future plans We will investigate the relationship between prenatal alcohol exposure and specific aspects of neurodevelopment at 6–8 years, including craniofacial shape, brain structure and function. The contribution of genetics and epigenetics to individual variation in outcomes will be examined in conjunction with national and international collaborations.

Strengths and limitations of this study

- The Asking Questions about Alcohol in Pregnancy cohort study was specifically designed to prospectively collect high-quality data on low to moderate prenatal alcohol exposure and relevant confounders to investigate the risk to offspring neurodevelopment.
- The children are being followed up for the third time at 6–8 years, using sensitive measures of neuropsychological function, 3D craniofacial photography and brain MRI.
- A biobank of birth samples and maternal and child buccal DNA enable investigation of the contribution of genetic and epigenetic factors to neurodevelopmental outcomes.
- Despite carefully designed questions, reporting bias will need to be considered in the interpretation of findings, especially around alcohol use.
- The generalisability of some findings will be limited to a general antenatal population of Caucasian women, from middle-income backgrounds and with a low-risk pregnancy.

INTRODUCTION

Alcohol crosses the placenta and is teratogenic.[1 2] Health guidelines around the world, including those developed by the Australian National Health and Medical Research Council, recommend that women who are either pregnant or planning a pregnancy abstain from drinking alcohol.[3] Alcohol can damage the developing fetal brain through oxidative stress, damage to the mitochondria and interference with the function of growth factors and neurotransmitters as well as through epigenetic changes which regulate gene activity.[2 4 5] The consequences can be devastating at a foundational stage of brain development, ultimately disrupting

neuronal proliferation and migration and glial functioning.[2]

High levels of alcohol exposure to the fetal brain can cause a spectrum of structural brain abnormalities, facial dysmorphology, neurological problems and neurodevelopmental impairments, collectively termed Fetal Alcohol Spectrum Disorder (FASD).[4 6–8] These effects have been replicated in animal models and are undisputed.[2]

Many pregnant women consume some alcohol during pregnancy, especially around the time of conception.[9–12] This is extremely concerning given the potential harms of prenatal alcohol exposure (PAE) to the developing fetus. Unplanned pregnancy is a common explanation for early pregnancy drinking, particularly for binge drinking exposure.[13] However, even after pregnancy awareness, a substantial proportion of women continue to drink at low to moderate levels,[9] sometimes with the knowledge that PAE has the potential to lead to lifelong disabilities in a child.[14] The lack of convincing evidence of harm from lower levels of PAE[15 16] and conflicting messages from health professionals concerning adverse effects of low to moderate PAE on the fetus are reasons given by some women for their decision not to abstain.[17]

The effects of PAE vary between individuals likely due to genetic, metabolic, nutritional, social and environmental factors as well as the timing, duration and dose of alcohol.[18] Human research has provided limited evidence that low to moderate PAE is detrimental to the offspring, with a recent systematic review reporting adverse effects on early child development in six studies, no effect in five studies, and a weak positive effect in two.[19] The authors concluded that conflicting findings following low PAE may in part be due to a lack of sensitivity for detecting some outcome measures, and inadequate accounting for confounding, environmental and social factors. Since this review was published, a secondary analysis of 9719 children from the Adolescent Brain Cognitive Development Study found that even children with low PAE demonstrated poorer psychological and behavioural outcomes at around 9–10 years of age.[20] The authors claimed their findings were robust because potential confounding factors were considered, and that stringent demographic matching procedures increased the plausibility of the findings, but while the study's sample size is impressive, collection of exposure and confounder information occurred retrospectively in preadolescence, raising questions around recall and accuracy.

The Asking Questions about Alcohol in Pregnancy (AQUA) prospective cohort study was designed to address the limitations in exposure measurement and collection of confounders, allowing for a robust investigation of the effects of common drinking patterns in pregnancy.[21]

The primary objective of this current follow-up of the cohort (*AQUA at 6*) is to assess neurodevelopment (neuropsychological functioning, brain structure and function and craniofacial shape) in a population-based cohort of children aged 6–8 years with respect to their PAE (none, low, moderate, high or binge level alcohol exposure), taking into account related maternal, child and socioenvironmental factors that may explain individual differences in outcome.

Hypotheses

1. Any PAE has the possibility of being associated with craniofacial changes (eg, mid-face, nose, lips and eyes), structural brain changes (eg, corpus callosum, basal ganglia, cerebellum) and subtle neuropsychological deficits (eg, motor, attention, executive function, memory and behaviour) at 6–8 years of age.
2. These PAE associations will be influenced by the timing and quantity of alcohol exposure, individual child and maternal characteristics (eg, genetics, nutrition, breastfeeding, maternal mental health) and socioenvironmental factors (eg, education, lifestyle, parenting style).
3. Craniofacial differences at 12 months of age will be associated with outcomes at 6–8 years, specifically (a) craniofacial shape, (b) brain structure and (c) neuropsychological functioning.

COHORT DESCRIPTION

The AQUA study comprises a cohort of mother/child dyads recruited from the general population in early pregnancy for longitudinal observation. All women with a singleton pregnancy, attending their first antenatal appointment before 19 weeks gestation, between 25 July 2011 and 30 July 2012, at one of seven public hospital recruitment sites in metropolitan Melbourne, Australia, were eligible to participate. Being 16 years or older and being able to read and write English were prerequisites for participation. The methods are described in detail in the original study protocol.[21] During pregnancy, women completed three questionnaires, (1) at recruitment (<18 weeks' gestation), (2) at 25 weeks' gestation and (3) at 35 weeks' gestation. After birth, questionnaires were sent at 12 and 24 months to women who had completed the three pregnancy questionnaires, and for whom complete PAE information was available (n=1570). An exposure representative subsample of 850 children were sequentially invited to have a 3D craniofacial photo taken at 12 months (517 images taken), and/or a neurodevelopmental assessment using the Bayley Scales of Infant and Toddler Development (Bayley-III) at 24 months (554 assessments completed).

The cohort of children was recruited again aged between 6 and 8 years, for further assessments, including longitudinal 3D analysis of craniofacial shape, state-of-the-art neuroimaging and standardised neuropsychological measures to assess neurodevelopmental status. Outcome measure details are provided in a dedicated section below.

Study design and procedures

Of the 1570 mother and child dyads from the original cohort, 55 mothers had withdrawn from the study. We excluded 108 who were lifetime alcohol abstainers

because our target population was children of mothers who normally drink some alcohol. Another 59 mothers were excluded who could not be classified because they abstained in the first trimester, then averaged an intake of less than one standard drink per week for the remainder of their pregnancy.[9] Therefore, in the *AQUA at* 6 follow-up study, 1348 mothers and children were invited to participate. Following the invitation to take part, a further six families were excluded from *AQUA at 6*, because of a recent oncology diagnosis in the child (n=3) or because of a later diagnosed condition impacting long-term development (one child with Down syndrome, one child with Dopa Responsive Dystonia and another child with Sanfilippo Syndrome). The final number of families eligible to participate was 1342.

Data were collected between June 2018 and April 2021.

Neuropsychological assessments and 3D craniofacial imaging were performed in specialist facilities at the Murdoch Children's Research Institute and Royal Children's Hospital (RCH) in Melbourne, Australia. For families unable to travel to the campus, the neuropsychological assessments were administered in the home, at school or another suitable facility such as a library meeting room. Externally assessed test results were obtained when the child had been recently assessed. A PAE-representative subset of children was sequentially invited to have a brain MRI scan, with a target number of 50 in each of three exposure groups: (1) no PAE, (2) PAE in trimester one only *and* (3) PAE throughout gestation. Primary caregivers (ie, the AQUA study mother in most cases) completed questionnaires online. This questionnaire was also offered to families whose child did not attend a neuropsychological assessment, but who still wished to take part.

For study participation, the neuropsychological assessment and/or questionnaire needed to be completed. All other aspects of the study were optional.

Impact of COVID-19

Following the COVID-19 pandemic, adaptations to the assessment procedures were necessary to comply with relevant institutional and government guidelines for a safe environment for study participants and assessors. Due to two government-mandated, state-wide lockdowns, face-to-face-assessments were suspended from 17 March to 24 June 2020 and again from 9 July to 20 October 2020. Outside these dates, face-to-face assessments were offered where possible, but with physical distancing measures and hygiene procedures in place to minimise risk of viral transmission. Online telehealth-style assessments via a video conferencing platform were also developed and offered from 12 June 2020, so that families were able to take part while remaining in their own home. The latter involved an abbreviated assessment as certain measures could not be administered using telehealth (eg, movement and coordination items) (online supplemental table 1). Families who took part in the telehealth-style assessment were invited to attend the hospital for a 3D craniofacial photo at a later date with the end to lockdown and when site visits became possible again.

Participation rates

Of the 1342 eligible families, 802 completed the minimum data required for participation (60%) and neuropsychological assessment data are available for 696 children (52%) (table 1). From commencement of the COVID-19 pandemic, 169 of the assessments were conducted in a telehealth format and another 73 in person with physical distancing in place. Following consent, we obtained externally assessed scores from the family's private psychologist for nine children, which in two instances were complemented by a partial assessment of the remaining tests.

Forty-one children who completed an assessment lived in another state of Australia, 23 of whom were visited by one of our assessors and 18 of whom completed a telehealth-style assessment. Another 14 children who lived overseas completed an assessment, three while visiting Melbourne and 10 via telehealth (data not shown).

Craniofacial photographs were obtained from 482 (36%) children. Participation rates in this aspect of the study were significantly impacted by the two Covid-19-related lockdown periods where site visits were not possible.

Most of the brain MRIs were obtained prior to the COVID-19 pandemic, with an additional four children able to take part in the time following the lockdowns, resulting in 146 scans (out of a proposed 150) being available for analysis.

Buccal swabs were collected from 427 children, either while attending an in-person assessment or via home collection using a mailed swab kit.

Of 540 eligible families did not take part in *AQUA at 6*: 308 opted out (23%); 71 for whom we had no current contact details (5%) and 161 who opted out passively either by not responding to any of our follow-ups or after initially expressing interest (12%).

Compared with those who did not take part, mothers participating in *AQUA at 6* were less likely to have been abstinent from alcohol or have smoked tobacco in pregnancy and were also less likely to be under 30 years of age at the time of birth. Mothers taking part were more likely to be tertiary educated at the time of initial recruitment and Caucasian (table 2).

Completion rates of previous postbirth study follow-ups in relation to *AQUA at 6* are presented in table 3.

Exposure assessment

PAE patterns were assessed in the original AQUA study.[9] Complete data on drinking frequency, amount and type of alcoholic drink(s) on each occasion were collected for 1570 participants via three questionnaires administered in pregnancy.

Timing of exposure

Maternal alcohol consumption data were reported for five stages of pregnancy: (1) 3 months before pregnancy;

Table 1 *AQUA at* 6 participation rates

	Eligible	Did not take part	Questionnaire completed	NP assessment	Craniofacial image	Brain MRI	Buccal swab
Invited	1348						
Excluded	6						
Unable to contact		71					
No final response to any follow-up*		161					
Opted out of *AQUA at 6*		271					
Withdrew from study		37					
Partial questionnaire only			12				
Questionnaire only†			94				
Full neuropsychological assessment			445‡	445	352	142	352
Post-COVID neuropsychological assessment			73	73	71	4	42
Tele-neuropsychological assessment			169§	169	58		32
Partial completion and/or external scores			9	9	*		*
Total	1342 (*100%*)	540 (*40%*)	802 (*60%*)	696 (*52%*)	482 (*36%*)	146 (*11%*)	427 (*32%*)

*Iincludes 49 mothers who agreed to the questionnaire but did not attempt to complete it.
†Includes two questionnaires where child attended the neuropsychological assessment, but was unable to complete this.
‡Includes one partially completed questionnaire.
§Includes one partially completed questionnaire.
AQUA, Asking Questions about Alcohol in Pregnancy.

Table 2 Demographics of *AQUA at* 6 participants compared with those who did not take part

	Participants n=802		Non-participants n=540	
	n (%)	n missing	n (%)	P value*
Prenatal alcohol exposure		0		0.002
Abstinent	**257 (32.0)**		219 (40.6)	
Any in trimester one	218 (27.2)		144 (26.7)	
Any throughout	327 (40.8)		177 (32.8)	
Maternal age at birth		0		<0.001
<30 years	**182 (22.7)**		195 (36.1)	
30–34 years	356 (44.4)		202 (37.4)	
35 years or over	264 (32.9)		143 (26.5)	
Maternal education (pregnancy)		2		<0.001
Secondary	**103 (12.9)**		135 (25.0)	
Diploma/trade	**179 (22.4)**		174 (32.2)	
Tertiary	**518 (64.8)**		231 (42.8)	
Socioeconomic background†		20		0.26
Lowest quintile (most disadvantaged)	51 (6.5)		27 (6.0)	
Second quintile	115 (14.7)		84 (18.7)	
Third quintile	167 (21.4)		101 (22.5)	
Fourth quintile	217 (27.8)		129 (28.7)	
Highest quintile (least disadvantaged)	204 (26.1)		96 (21.4)	
Lives overseas	28 (3.3)		12 (2.7)	
Maternal ethnicity Caucasian		1		0.002
Yes	**697 (87.0)**		437 (80.9)	
Maternal pre-pregnancy BMI		25		0.54
Under/normal weight (<25)	511 (65.8)		327 (63.0)	
Overweight (25 to ≤30)	144 (18.5)		108 (20.8)	
Obese (>30)	122 (15.7)		84 (16.2)	
Pregnancy planning		3		0.13
Yes	622 (77.9)		401 (74.3)	
Primipara		0		0.50
Yes	387 (48.3)		250 (46.3)	
Maternal smoking in pregnancy		0		0.001
Yes	**118 (14.7)**		116 (21.5)	
Child sex		0		0.57
Male	410 (51.1)		262 (49.5)	
Child born preterm (<37 weeks)		0		0.09
Yes	35 (4.4)		14 (2.6)	
Child born small for gestational age[e]		15		0.50
Yes	50 (6.4)		28 (5.4)	

*Pearson's χ^2 test; boldface=statistically significant difference between participants and non-participants.
†Based on the Index of Relative Socio-economic Disadvantage: a general socio-economic index summarising and ranking a range of information about the economic and social conditions of people and households within a small geographic area (Statistical Area 1). The index was calculated from the 2016 Census of Population and published by the Australian Bureau of Statistics.
AQUA, Asking Questions about Alcohol in Pregnancy; BMI, body mass index.

(2) trimester 1 prepregnancy aware, (3) trimester 1 post-pregnancy aware, (iv) trimester 2 and (v) trimester 3. The mean (SD) gestational age at pregnancy recognition was 4.9 (1.5) weeks.[9]

Levels of exposure

Women were asked to use a pictorial drinks guide, listing common types and volumes of alcoholic drinks, to identify their 'usual' pattern of drinking, with provision for up to five types of alcoholic drink. For each beverage identified, they were asked how often they usually drank this type of alcohol and how many drinks they usually consumed on each occasion. Women were also asked if there were any 'special occasions' (or difficult times) when they consumed more alcohol than usual, the frequency of these occasions, the drink types and the number of drinks per occasion. Estimates from 'special occasions'

Table 3 *AQUA at* 6 participation and availability of data from previous postbirth follow-ups

		Participation in *AQUA at 6*						
		Overall participation	*Detailed participation*					
	Total	Did not take part	Took part	Questionnaire only	Neuropsych assessment	Craniofacial image	Brain MRI	Buccal swab
	540	**802**	**106**	**696**	**482**	**146**	**427**
Biospecimen collection at birth								
Placental biopsy	**225**	76	149	14	135	99	40	94
Cord blood	**188**	65	123	11	112	83	35	80
Neonatal buccal swab	**646**	212	434	52	382	271	93	250
Participation at 12 months								
Questionnaire completed	**1102**	368	734	95	639	446	135	395
Craniofacial image	**512**	76	436	26	410	314	103	221
Participation at 24 months								
Questionnaire	**945**	254	691	85	606	431	131	378
Neuropsych assessment	**551**	78	473	28	445	338	112	297

AQUA, Asking Questions about Alcohol in Pregnancy.

were combined with information from 'usual' alcohol consumption to calculate a maximum weekly intake.[9] The number and types of drink reported by women were first converted to standard drinks before calculating the amount of absolute alcohol in grams (gAA) consumed. One standard drink in Australia is equal to 10 gAA (online supplemental resource).

Alcohol abstinence throughout pregnancy (but not lifetime abstainer) was defined as the unexposed control group—no PAE.

Summarised exposure group data for the *AQUA at* 6 eligible cohorts (ie, no PAE; PAE in trimester 1 only; PAE throughout gestation) and participation in the study's core components are presented in table 4. The PAE group distribution in the neuropsychological assessment and 3D craniofacial image data differed marginally from that in the eligible cohort, due to somewhat higher rates of participation in the 'any PAE throughout pregnancy' groups.

In addition to this broad exposure classification, group-based trajectory modelling (GBTM) will be used as a data-driven method of classifying the temporal, continuous PAE data for all *AQUA at* 6 analyses. GBTM can be used to objectively identify alcohol consumption trajectories arising directly from the source data without the need for predetermined classification,[22] which has the potential to result in a more accurate and nuanced representation of the exposure to the fetus.

Outcome measures

Neuropsychological assessment

Children underwent a 3–4-hour neuropsychological assessment by trained psychologists blinded to PAE exposure and previous assessments (table 4). The measures used were validated, well-established and sensitive to brain insult and were based on measures identified as important to identify neurodevelopmental impairments that are reported in FASD research and included in diagnostic guidelines.[7 23 24] They include general intelligence (intelligence quotient; IQ), attention, executive function, memory and learning, language and motor function. Neuropsychological assessments were complemented by information collected via a parent-report questionnaire using validated measures (table 5).

Craniofacial imaging

Craniofacial imaging of the study child was undertaken by an experienced medical photographer using a 3dMD 7-pod system (3dMD corporation Atlanta, Georgia), which captures a full 360° image of the head (face and cranium). To ensure that images were unobscured by hair and to capture the shape of the neurocranium, a tight-fitting stocking was placed over the cranial vault. Images were captured in less than 1 s and available for review within 3 min. The photographer and craniofacial image analyst were blinded to the children's PAE.

To represent the entire surface of the cranium and face, a spatially dense array of 69 587 points on an age-matched

Table 4 *AQUA at* 6 participation by summarised PAE group

		Exposure group		
		No PAE	Any PAE in trimester 1 only	Any PAE throughout pregnancy
	N	N (%)	n (%)	n (%)
Eligible cohort	1342	476 (35.5)	362 (27.0)	504 (37.6)
Neuropsych assessment	696	223 (32.0)	182 (26.1)	291 (41.8)
3D craniofacial image	482	152 (31.5)	121 (25.1)	209 (43.4)
Brain MRI	146	42 (28.8)	45 (30.8)	59 (40.4)
Questionnaire only*	106	34 (32.1)	36 (34.0)	36 (34.0)

*Includes 12 partially completed questionnaires.
AQUA, Asking Questions about Alcohol in Pregnancy; PAE, prenatal alcohol exposure.

template face (pseudo landmarks) is automatically mapped onto each target image by a 3D surface registration algorithm. This warps the shape of the template into the shape of the target face, sampling each face at corresponding locations across the entire surface. A partial least squares regression-based hypothesis testing framework,[25] suitable for highly multivariate shape data, can be applied to test for group differences, while adjusting for covariates. This can be done on the whole face or on localised facial segments such as the eyes, nose and philtrum.[26]

Brain MRI

Brain imaging was undertaken at the RCH, Melbourne, using a 3 Tesla Siemens MAGNETOM Prisma scanner. The imaging sequences are listed in table 6, with total time in the scanner around 45 min. Children in the MRI subgroup were scanned on the same day or within 2 weeks of their neuropsychological assessment. To ensure high compliance and quality images, children completed a preparatory session with a mock MRI scanner prior to their MRI appointment.

Postacquisition MRI analysis includes investigation of: (1) regional brain volumes (66 cortical, 14 subcortical) and cortical morphology (thickness, curvature and sulcal depth) using FreeSurfer V.7[27]; (2) volume and morphology of the corpus callosum, hippocampus, basal ganglia and cerebellum, regions hypothesised to be

Table 5 Neuropsychological assessments

Outcome domain	Scale/subtest
Psychologist assessed (direct assessment)	
General intelligence	Core subtests of the Wechsler Intelligence Scale for Children[50]
Language	WISC-V Verbal Comprehension Index[50]
Academic functioning	Wechsler Individual Achievement Test subtests. Literacy is assessed using the word reading and spelling subtests, while mathematics is assessed using the numerical operations subtest[51]
Attention	Test of Everyday Attention for Children-Version 2 subtests (*age <8 years*: Balloon Hunt, Barking, Sustained Attention to Response Task and Simple Reaction Time; *age ≥8 years*: Hector Cancellation, Vigil, Sustained Attention to Response Task and Simple Reaction Time)[52]
Working memory	Digits Recall, Blocks Recall and Backward Blocks Recall subtests of the Working Memory Test Battery for Children[50]
Cognitive flexibility	Contingency Naming Test (trials 1–3)[53 54]
Episodic memory	The California Verbal Learning Test—Children's version[55]
Motor functioning	Movement Assessment Battery for Children[56]
Parent report (indirect assessment)	
Attention	Attention Deficit Hyperactivity (ADHD) Rating Scale 5[57]
Emotional and behavioural status	Strengths and Difficulties Questionnaire[58]
Executive function behaviours	Behavior Rating Inventory of Executive Function-Second Edition questionnaire[59]
Autism symptoms	Social Communication Questionnaire[60]
Movement and coordination	Developmental Coordination Disorder Questionnaire[61]

Table 6 MRI sequences	
1	T1- weighted multi-echo MP-RAGE* images with 0.9 mm^3 isotropic voxels (IVs) and echo planar image-navigated prospective motion compensation
2	Multi-shell simultaneous multi-slice echo planar diffusion images (b=750, 25 gradient directions; b=2000, 30 directions; and b=3000 s/mm2, 45 directions) with 1.5mm^3 IVs and matching reverse phase encoding sequences
3	3D T2-weighted turbo spin echo images with 0.9mm^3 IVs
4	Multiband, multi-echo gradient recalled echo planar resting state functional MRI images with 2.4 mm^3 IVs, with prospective acquisition correction and reverse phase encoding images

*Magnetization Prepared - RApid Gradient Echo

particularly important given previous FASD research[1]; (3) white matter microstructural organisation and maturity using (a) myelin mapping by applying the T_1–T_2 ratio,[28] (b) the spherical mean technique, which can estimate diffusivity and neurite density from diffusion MRIs without influence from crossing fibres[29] and (c) fixel-based analyses, a fibre-based analysis of apparent fibre density[30]; (4) whole-brain white matter tract analyses using tract-specific analyses[31] as well as detailed examination of the corpus callosum, anterior–posterior fibre bundles and cortico-spinal tracts using constrained spherical deconvolution tractography,[32] given their importance based on previous FASD research[33]; (5) structural connectivity is examined using constrained spherical deconvolution-based white matter fibre tractography to find connections between FreeSurfer-derived brain regions. Using graph theory analyses, metrics such as global and local efficiency, small worldness and rich club organisation will be produced to investigate the efficiency, integration or segregation of brain networks[34] and (6) functional connectivity analyses will also be done by applying independent cocmponent analyses to resting state images, to find temporal correlations in spontaneous blood oxygen level-dependent signal between brain regions.[35]

Australian Early Developmental Census

Consent was sought to link *AQUA at* 6 children who attended their first year of school in 2018 to the Australian Early Developmental Census (AEDC),[36] and 93% of mothers with an eligible child consented to this data linkage. The AEDC is undertaken every 3 years using the Australian version of the Canadian Early Development Instrument, with the most recent year being 2018.[37] The instrument consists of 100 questions and is completed by teachers on the basis of at least 1 month's knowledge of the child. It covers the five domains of physical, social, emotional, language and cognitive development as well as data on special needs. Children falling below the 10th percentile in any domain are considered developmentally 'vulnerable' in that area, children falling between the 10th and 25th percentile are considered developmentally 'at risk', and all other children are considered to be 'on track'. Approval for linkage to relevant data items at an individual (micro) level will be obtained from the AEDC custodians and the linkage will be conducted independently by an authorised Data Linkage Agency.

Confounders and modifiers

During the original 2011–2014 AQUA study, extensive data were collected on factors that may confound or modify the relationship between PAE and child outcomes. These included maternal obstetric history and complications, maternal nutrition, medication and supplementation, breastfeeding, maternal and paternal alcohol, tobacco and illicit drug use, maternal mental health, education and other sociodemographics, family relationships and parenting.[21] Updated relevant information on demographic and socioenvironmental factors was collected from the child's primary caregiver (table 7).

Biospecimens

A comprehensive biobank of maternal and child DNA from the AQUA study exists, comprising extracts from placental biopsies, cord blood mononuclear cells and maternal and neonatal buccal swabs to investigate how genetic, epigenetic and environmental factors interact with PAE to explain individual variation in child outcomes.[21] This biobank was extended for future

Table 7 Demographic and socio-environmental factors collected by parent report	
Domain	**Questions**
Demographics	Ethnic group mother and child, child language spoken at home, mother high school education and post school training, mother work status, healthcare, financial situation, partner education and work status
Child health	Overall health, Child Special Health Care Needs Screener (CSHCN),[62] professional assistance and support
Parenting	Child Rearing Questionnaire,[63] Parental Expectations and Limitations,[63] Hostile Parenting[63]
Mother health and lifestyle	Depression Anxiety Stress Scales (DASS-21),[64] Alcohol Use Disorder Identification Test—Consumption (AUDIT-C),[65] tobacco use, illicit substance use
Family and relationships	Marital status, family structure, number of children living in household, McMaster Family Functioning Subscale,[66] family support,[67] couple relationships,[67 68] domestic violence,[69] partner alcohol (AUDIT-C), partner tobacco, partner illicit substance use

collaborative investigations in this area through the collection of 427 child buccal swabs in 6–8-year olds.

Findings to date

In the original cohort, there were 1570 mother–child pairs, of whom 59% of mothers reported drinking alcohol during pregnancy and 19% reported at least one episode of binge drinking prior to pregnancy recognition.[9] The study found an association between low to moderate PAE and craniofacial shape in the children aged 12 months, with differences concentrated around the nose, eyes and mouth.[38] This has potential clinical implications given that development of the face parallels, and is controlled by, the brain. However, at 2 years of age, no adverse association was detected between child neurodevelopment and low to moderate PAE using the Bayley Scales of Infant and Toddler Development (Bayley-III).[39] Given that measures of early development are only moderately predictive of school-aged outcomes[40] and do not reliably assess higher order cognitive and motor functions,[41 42] this school-aged follow-up of this cohort is essential to determine any long-term effects of low to moderate PAE and binge episodes.

POWER CALCULATIONS

Regarding the neuropsychological outcomes, using a two-sided 0.05 significance level, with 52% participation rate, we have 80% power to detect a small but clinically significant effects (Cohen's f=0.12). As an example, for full-scale IQ as effect of this magnitude would translate to a mean difference of 3.6 IQ points between the three major PAE groups. In terms of the MRI data, an important measure of interest is brain volume. Assuming a mean intracranial volume of around 1414 cubic cm (cc) (SD=99 cc),[43] which is based on typically developing 7-year-old children in Melbourne, with a sample size of 146, we will have 80% power to detect a difference of 54 cc in total brain volume between groups (medium effect size f=0.26). This volumetric reduction represents an effect of 0.54SD or 4% of the total volume. Traditional power calculations are not possible for craniofacial analysis, where the outcome measure is many thousands of point coordinates. In our 1-year analysis, we detected differences ($p<0.05$) using a control group of 89 and PAE groups of approximately 40 images,[38] which gives us confidence that our proposed analyses will be sufficiently powered.

Strengths and limitations

This study has several unique features that will enable the relationship between PAE and neurodevelopment to be addressed rigorously. First, AQUA has very detailed assessments of drinking patterns in the periconceptional period and during pregnancy, including timing, frequency and quantity of alcohol consumption. Second, AQUA is a large representative cohort of pregnant women from the community, recruited for the specific purpose of assessing common drinking patterns. Previous studies have tended to focus on high-risk groups of substance users, risking selection bias or take advantage of crude measures collected during large epidemiological studies.[16 44 45] Third, at each phase of data collection, we have gathered information relating to important modifiers and confounders including nutrition, mental health, physical health and socioeconomic status. Adjustment for these factors is essential given their relationship to both drinking behaviour and child development. Finally, AQUA uses sensitive measures targeting specific areas of neurodevelopment informed by FASD research including longitudinal analysis of craniofacial shape, brain structure and function and neuropsychological functioning. Of importance, our investigations include whether early craniofacial changes are predictive of later neuropsychological impairments, and whether PAE is associated with a common pattern of neural abnormalities demonstrated on MRI.

A limitation of any study measuring PAE is that there are currently no validated objective measures to quantify low to moderate exposure,[46] and researchers depend on accurate maternal recall and reporting. In order to maximise accuracy of reporting in the AQUA study, we involved pregnant women in the development of the alcohol consumption questions to be used in the AQUA study.[47] This work indicated that women who attend general antenatal care would answer as truthfully as possible, due to their vested interest in understanding what may be considered normal, non-risky pregnancy drinking habits. The opportunity to report heavy or binge drinking on 'special occasions' yielded important information on early gestation exposures, information that might not have been reported in more general questioning.[9]

The validity of some covariates such as maternal lifestyle and family relationships may also be subjected to reporting bias due to a desire to provide socially acceptable responses. Findings will need to be interpreted in the context of existing literature on the causal relationships between such variables and child neurodevelopment.

Finally, in instances when direct neuropsychological assessment of the child was not possible, we depended on indirect measures (eg, maternal report) to determine developmental progress, which is subjective and may introduce informant bias.

In summary, a significant proportion of pregnant women does not adhere to health policy guidelines and drink some alcohol, potentially putting thousands of children at risk for life-long neurodevelopmental impairments. No safe level of alcohol consumption in pregnancy has been established, and women's drinking behaviour in part reflects the lack of evidence to support health professional advice that women who are pregnant should not drink alcohol. Findings from this study will have an impact from a preventative health perspective, providing strong evidence on the consequences of low to moderate and binge-level PAE, strengthening the messages provided to the public through education and health promotion campaigns.

COLLABORATIONS

As with our 12 month follow-up, we are collaborating with two experts in 3D morphometric analysis of image data: Drs Peter Claes and Harold Matthews from KU Leuven in Belgium. The collaboration aims to develop new approaches to undertake our craniofacial analysis and to interpret these results.

Other collaborations to date have arisen from our interest in epigenetics, specifically, the association between PAE and DNA methylation and its role as a mediator of neurodevelopment and FASD. We are contributing data to the Pregnancy And Childhood Epigenetics consortium as part of their meta-analysis project studying early life environmental impacts on human disease using epigenetics. The consortium is based at the US National Institute of Environmental Health Sciences and includes researchers from around the world. (https://www.niehs.nih.gov/research/atniehs/labs/epi/pi/genetics/pace/index.cfm).

We are also collaborating with the lab of Professor Michael Kobor, Centre for Molecular Medicine and Therapeutics, BC Children's Hospital Research Institute, The University of British Columbia. The Kobor lab recently developed a paediatric epigenetic clock (PedBE) using buccal epithelial swabs (https://github.com/kobor-lab/Public-Scripts/). The collaboration will generate epigenetic and genotypic data from our child buccal DNA to contribute to their project investigating the extent to which the PedBE clock informs on child development across diverse populations and sex. Another collaboration in this area of study is with a team at the Telethon Institute, Western Australia, led by Dr David Martino. The AQUA study is contributing Epigenome-wide Association study data from buccal epithelial swabs for this project, which aims to identify DNA methylation biomarkers of PAE in a controlled murine experiment, with replication in existing methylation data sets from human infants with well characterised PAE exposure patters and children diagnosed with FASD (https://www.telethonkids.org.au/contact-us/our-people/m/david-martino/).

The AQUA study welcomes new collaborations with other investigators and has actively engaged in collaborative data sharing projects. Interested investigators should contact the project manager Evi Muggli (evi.muggli@mcri.edu.au) to obtain additional information about the study and referral to the appropriate chief investigators for the discussion of collaborative opportunities.

The AQUA study has obtained participant consent to have their data included in other ethically approved studies in related areas of research.

FURTHER DETAILS

Data management

All study data are collected and managed using REDCap electronic data capture tools hosted at The Murdoch Children's Research Institute in Melbourne, Australia.[48 49] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing (1) an intuitive interface for validated data capture, (2) audit trails for tracking data manipulation and export procedures, (3) automated export procedures for seamless data downloads to common statistical packages and (4) procedures for data integration and interoperability with external sources. REDCap is also used to facilitate tracking and scheduling of all communication with participants. Electronic raw and derived data, including longitudinal data from the first phase of the AQUA study, will be stored on a restricted server and curated by the project manager (EM).

Ethics approval and consent to participate

All personal information of potential and enrolled participants was collected only for research purpose and will be kept in strict confidentiality by the investigators and project staff. Personal information is stored separately from other research data and will be linked using the family's study ID, which was assigned at enrolment of the pregnant mother in the first phase of the AQUA study. Hard copy materials are kept in locked compartments and electronic records are stored with password encryption. All hard copy and electronic data are stored until child participants are 25 years of age or for 15 years after the study has been completed, whichever is later.

Ethics approval and consent to participate in previous waves of the AQUA study

Ethical oversight of the cohort's recruitment and prebirth and neonatal follow-ups was provided by the Eastern Health Research and Ethics Committee (E54/1011) and the Human Research Ethics Committees of Mercy Health (R11/14), Monash Health (11071), the Royal Women's Hospital (11/20) and the Royal Children's Hospital (31055). The latter also included approval of all procedures pertaining the 12-month and 24-month postpartum follow-ups. Families who have not actively withdrawn their consent to participate are ongoing study participants and their data may be included in future analyses by the project team if they are deemed to be in line with information that was provided to participants at the time of consent.'

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Contributors EM, JH, EE, AP, DT, AJS, DF, SL, SH and PJA were involved in the conception and in the design of the study and have made substantial contributions to the planning of the study. EM drafted the manuscript and is responsible for the overall content. EM, JH, EE, AP, DT, AJS, DAF, SL, SH and PJA critically revised manuscript drafts and agreed with the final version of the manuscript.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Human Research Ethics Committee of the Royal Children's Hospital, Melbourne, Australia, approval number is 38025. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. The datasets generated and/or analysed during the current study will not be publicly available as the study is ongoing. The raw data supporting the conclusions of future manuscripts will be made available to the journal's supplementary material by the authors, if requested. *AQUA at 6* study families have the option to consent for their data to be used in future related and ethically approved projects. Following study completion, data will be available from the corresponding author upon reasonable request to such projects.

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Supplementary Table Telepsychology modifications to the in-person assessment protocol

Outcome domain	Assessments (in-person)	Assessment modification (telepsychology)
General intelligence	Core subtests of the Wechsler Intelligence Scale for Children (WISC-V).	Block Design, Coding and Symbol Search are not administered online. All other primary subtests are administered verbally or via screen-mirroring.
Language	Verbal Comprehension Index of the WISC-V.	No modification, tests administered verbally.
Academic functioning	Subtests from the Wechsler Individual Achievement Test (WIAT-III). Literacy is assessed using the word reading and spelling subtests, while mathematics is assessed using the numerical operations subtest	Numerical Operations are administered online as hard-copy response booklet cannot be adapted digitally. Word Reading stimulus is screen-shared, and child gives verbal responses. No modifications to Spelling, child writes response on paper and shows examiner.
Attention	Test of Everyday Attention for Children-Version 2 (TEA-Ch2) subtests (*age <8 years*: Balloon Hunt, Barking, Sustained Attention to Response Task and Simple Reaction Time; *age ≥8 years*: Hector Cancellation, Vigil, Sustained Attention to Response Task and Simple Reaction Time)	Balloon Hunt, Hector Cancellation, Simple Reaction Time and Sustained Attention to Response Task will not be administered online as they cannot be adapted for remote administration. [1]
Working memory	Digits Recall (WISC-V), Blocks Recall and Backward Block Recall subtests of the Working Memory Test Battery for Children (WMTB-C)	Block recall requires a block board and is not administered online.
Cognitive flexibility	Contingency Naming Test (CNT 1-3).	CNT stimulus sheet is screen-shared, and child gives verbal responses.
Episodic memory	The California Verbal Learning Test – Children's version (CVLT-C)	No modification, tests administered verbally.
Motor functioning	The Movement Assessment Battery for Children (MABC2).	The MABC2 is not administered online.

Asking Questions about Alcohol in pregnancy study (AQUA): Prenatal alcohol consumption assessment

ALCOHOL CONSUMPTION QUESTIONS

Using the code(s) provided in the Drinks Guide (for drinks guide see page 3), please complete the table below, including;

a) what type of drink(s) you usually drank in this period (for time points see page 2),

b) how often you usually drank this type of alcohol (for each), and

c) how many of these would you usually drink on each occasion (for each type of drink).

	Code for drink type	Frequency	Number per occasion
1.		☐ less than once a month ☐ 1 to 2 days per month ☐ 1 to 2 days per week ☐ 3 to 4 days per week ☐ 5 or more days per week	☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
2.		☐ less than once a month ☐ 1 to 2 days per month ☐ 1 to 2 days per week ☐ 3 to 4 days per week ☐ 5 or more days per week	☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
3.		☐ less than once a month ☐ 1 to 2 days per month ☐ 1 to 2 days per week ☐ 3 to 4 days per week ☐ 5 or more days per week	☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
4.		☐ less than once a month ☐ 1 to 2 days per month ☐ 1 to 2 days per week ☐ 3 to 4 days per week ☐ 5 or more days per week	☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
5.		☐ less than once a month ☐ 1 to 2 days per month ☐ 1 to 2 days per week ☐ 3 to 4 days per week ☐ 5 or more days per week	☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks

Did you ever drink more than you would normally have done (as you described above), for example on special occasions or during difficult times?

☐ no

☐ yes

Approximately, how many times did this occur during this period? (please provide number of occasions)

☐☐ occasions

Using the code(s) provided in the Drinks Guide, please complete the table below to show;

a) what type of drink(s) you usually drank on these special occasions or during difficult times, and

b) how many of these would you usually drink on each occasion (for each type of drink)

	Code for drink type	Number per occasion
1.		☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
2.		☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
3.		☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
4.		☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks
5.		☐ less than 1 drink ☐ 1 to 2 drinks ☐ 3 to 4 drinks ☐ 5 to 6 drinks ☐ 7 or more drinks

If '7 or more', what was the maximum number? (please provide maximum number of drinks on one occasion)

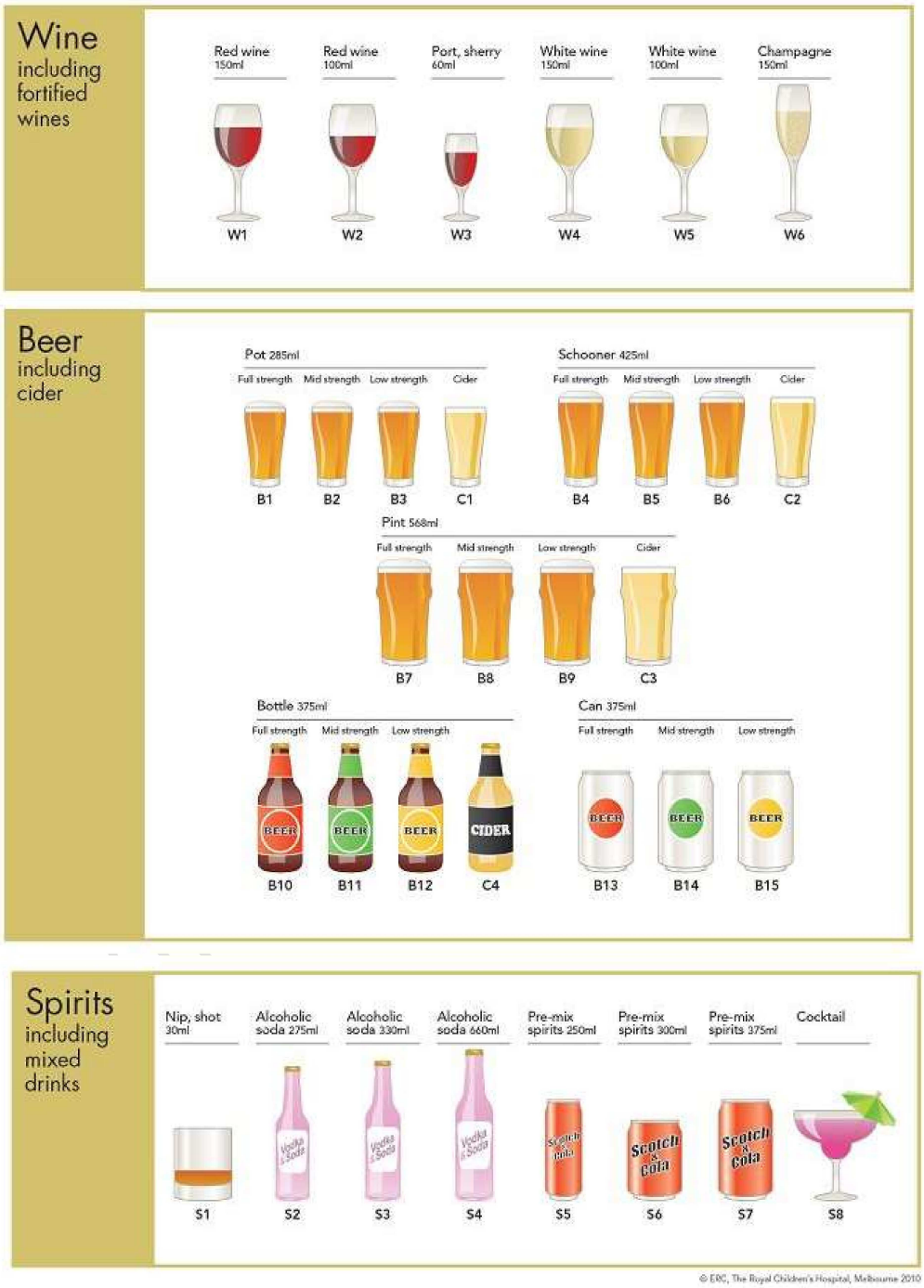
☐☐ drinks

TIME POINTS ADMINISTERED, INCLUDING PERICONCEPTION

These questions were administered at each trimester to collectively cover 5 time periods:

1) In the 3 months before you became pregnant
2) Since you became pregnant, but before you knew you were pregnant
3) Since you found out you were pregnant, to the end of your 13th week of pregnancy
4) Since your 14th week of pregnancy to the end of your 26th week of pregnancy
5) Since your 26th week of pregnancy (administered at around 35 weeks gestation)

DRINKS GUIDE



ALCOHOL CONTENT KEY TO AQUA STUDY DRINKS GUIDE

Drink type	Code	Volume ml	ABV[a] %	Std drinks[b] n	Total gAA[c]
Red wine, large glass	W1	150	13.5	1.5	15
Red wine, small glass	W2	100	13.5	1.0	10
Port, sherry, small glass	W3	60	17.5	0.9	9
White wine, large glass	W4	150	11.5	1.5	15
White wine, small glass	W5	100	11.5	1.0	10
Champagne, large glass	W6	150	12.0	1.5	15
Beer, full strength, pot	B1	285	4.8	1.1	11
Beer, mid strength, pot	B2	285	3.5	0.8	8
Beer, low strength, pot	B3	285	2.7	0.6	6
Cider, pot	C1	285	4.8	1.1	11
Beer, full strength, schooner	B4	425	4.8	1.6	16
Beer, mid strength, schooner	B5	425	3.5	1.2	12
Beer, low strength, schooner	B6	425	2.7	0.9	9
Cider, schooner	C2	425	4.8	1.6	16
Beer, full strength, pint	B7	568	4.8	2.1	21
Beer, mid strength, pint	B8	568	3.5	1.6	16
Beer, low strength, pint	B9	568	2.7	1.2	12
Cider, pint	C3	568	4.8	2.1	21
Beer, full strength, bottle	B10	375	4.8	1.4	14
Beer, mid strength, bottle	B11	375	3.5	1.0	10
Beer, low strength, bottle	B12	375	2.7	0.8	8
Cider, bottle	C4	375	4.8	1.4	14
Beer, full strength, can	B13	375	4.8	1.4	14
Beer, mid strength, can	B14	375	3.5	1.0	10
Beer, low strength, can	B14	375	2.7	0.8	8
Cider, can	C5	375	4.8	1.4	14
Spirits, nip/shot	S1	30	40	1.0	10
Alcoholic soda, small bottle	S2	275	5.0	1.1	11
Alcoholic soda, mid bottle	S3	330	5.0	1.2	12
Alcoholic soda, large bottle	S4	660	5.0	2.6	26
Pre-mix spirits, small can	S5	250	5.0	0.9	9
Pre-mix spirits, mid can	S6	300	5.0	1.1	11
Pre-mix spirits, large can	S7	375	5.0	1.4	14
Cocktail, glass	S8	n/a	n/a	2.5	25

[a] ABV: Approximate alcohol content by volume, based on the standard drinks guide published by the Australian Government
[b] STD drinks: based on the standard drinks guide published by the Australian Government.
[c] gAA: total grams of absolute alcohol contained in one drink. One standard drink in Australia is equal to 10 gAA.

(https://www.health.gov.au/health-topics/alcohol/about-alcohol/standard-drinks-guide)