Prevalence, clinical investigation, and   management of gallbladder disease in Rett syndrome

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ABBREVIATIONS

ARSD	Australian Rett Syndrome Database
InterRett	International Rett Syndrome Phenotype Database
RTT	Rett syndrome

[**ABSTRACT** (198 words)

AIM This study determined the prevalence of cholelithiasis and/or cholecystectomy in Rett syndrome, described gallbladder function in a clinical cohort, and identified recommendations for assessment and management of gallbladder disease.

METHOD The incidence rate of cholelithiasis/cholecystectomy was estimated from data describing 270 and 681 individuals with a pathogenic *MECP2* mutation in the Australian Rett Syndrome Database and the International Rett Syndrome Phenotype Database respectively. Gallbladder function in 25 females (age 3y 5mo-47y 10mo) with Rett syndrome was evaluated with clinical assessment and ultrasound of the gallbladder. The Delphi technique was used to develop assessment and treatment recommendations.

RESULTS The incidence rate for cholelithiasis and/or cholecystectomy was 2.3 (95% CI 1.1 to 4.2) and 1.8 (95% CI 1.0 to 3.0) per 1000 person-years in the Australian and International Databases respectively. The mean contractility index of the gallbladder for the clinical sample was 46.5% [SD 38.3%], smaller than for healthy individuals but similar to children with Down syndrome, despite no clinical symptoms. After excluding gastroesophageal reflux gallbladder disease should be considered as a cause of abdominal pain in Rett syndrome and cholecystectomy recommended if symptomatic.

INTERPRETATION Gallbladder disease is relatively common in Rett syndrome and should be considered in the differential diagnosis of abdominal pain in Rett syndrome.

What this paper adds

- Gallbladder disease occurs more frequently in those with Rett syndrome compared with the general population.

- Smaller gallbladder volumes and decreased contractility were found in Rett syndrome compared with healthy controls similar to those with Down syndrome.

- Gallbladder disease should be considered in the differential diagnosis of abdominal pain when it presents in this disorder.

Introduction
Rett syndrome (RTT) is a severe neurodevelopmental disorder caused by mutations in the X-linked methyl-CpG-binding-protein-2 (*MECP2*) gene.[1] After a period of apparently normal early development, there is a period of regression including loss of purposeful hand use

and/or language skills, and the development of distinctive hand stereotypies and impaired gait. There are also supportive criteria including poor growth, respiratory irregularities, and the development of scoliosis.[2] Many factors contribute to poor growth in RTT including altered oropharyngeal dysfunction and reduced food intake,[3,4] and problems with gastroesophageal motility such as reflux and constipation.[5] Dysmotility may result from absent primary or secondary intestinal waves and the presence of tertiary intestinal waves as observed during videofluoroscopy, with concurrent delayed gastric emptying and atony.[3] In a recent questionnaire-based survey in the USA (n=983), feeding problems were reported in 81% and gastrointestinal problems in 92% of individuals.[6] Gastrointestinal dysfunction in RTT contributes to clinical severity and impacts the daily lives of the females and their caregivers.[6]

In general, gallstones are rare in childhood and adolescence, with estimated prevalence between 0 and 2% in the small number of studies undertaken.[7-12] However, there appears to be a higher prevalence in particular populations of children, such as those with Down syndrome with reported prevalences up to 25%.[13,14] Clinical studies in Down syndrome indicate that the gallbladder is small with poor emptying after a meal, suggestive of hypomotility.[15] Table I summarises the limited available literature relating to the prevalence of gallbladder disease in the general paediatric population and those with Down syndrome. In the US family survey, gallbladder disease was reported by parents of 3% of females with RTT[6] and has also been noted in single case reports of a male[16] and a female[17] with RTT. Similar to Down syndrome, hypomotility of the gastrointestinal system in RTT could result in more frequent occurrence of gallbladder disease in comparison with the general paediatric population. However, the population-based prevalence of gallbladder disease in RTT remains unknown.

Gallbladder disease can be a significant medical problem associated with severe abdominal pain but gallstones can remain asymptomatic. Moreover, 17% of children with gallstones detected with ultrasonography were symptom-free and 24% presented with non-specific abdominal pain.[8] Given the severe degree of intellectual impairment associated with RTT and the frequent difficulties carers and clinicians often have in identifying the cause of pain and other symptoms,[18] it is important to further investigate the prevalence of gallbladder disease in this population. It is also important to provide clinicians with recommendations to assist them in the timely management of this often forgotten gastrointestinal disorder.

There are three parts to this study. Firstly, we investigated the occurrence of gallbladder disease using a population-based register and a large international sample;

secondly, we assessed gallbladder function in a clinic sample of females with RTT; and, thirdly, we report clinical recommendations for the assessment and management of gallbladder disease in RTT developed using consensus methods.

METHOD

Part 1: Occurrence of gallbladder disease

Data were sourced from two databases: the Australian Rett Syndrome Database (ARSD)[19] and the International Rett Syndrome Phenotype Database (InterRett).[20] Initially established in 1993, the ARSD is an ongoing systematically ascertained, longitudinal population-based register of all Australian Rett syndrome cases born since 1976.[19] For this study eligible cases were those who fulfilled the revised diagnostic criteria for RTT,[2] had a pathogenic *MECP*2 mutation, and whose caregivers had returned a questionnaire on registration and one or more biennial follow-up questionnaires. Age at questionnaire return, mutation type, and most recent height and weight were extracted from the database along with data to describe whether the individual had developed cholelithiasis, cholecystitis, or biliary dyskinesia, and when diagnosis was made. Contact was made with caregivers of cases with a gallbladder disorder to confirm or refine the diagnosis.

InterRett was established in 2003[20] and is a phenotype database that contains information on RTT cases from multiple countries around the world. This analysis was restricted to individuals with a confirmed pathogenic mutation whose caregivers had completed a questionnaire. Specifically, families were asked if their child was diagnosed with any gallbladder or biliary disease. Age and mutation details were extracted from the database. A binary variable was created to indicate the occurrence or otherwise of a diagnosis of gallbladder disease. To facilitate calculation of the incidence rate of gallbladder disease, the observation time or years of follow-up was determined for each individual.For those who had had gallbladder disease, the observation time was the age at diagnosis and, for the remainder, the age at return of last family questionnaire or age at death.

Statistical analyses

Incidence rates of gallbladder disease for the ARSD and InterRett cohorts were estimated by dividing the number of newly diagnosed cases by the sum of observation times for each individual at risk. Confidence intervals (CI) for incidence rates were calculated using the Poisson exact method.

Part 2: Gallbladder function in a clinic sample

This component of the study was performed at the Department of Paediatrics and Adolescent Medicine of the Medical University Vienna. Patients were recruited through an advertisement in the local newsletter of the Austrian Rett Syndrome Society. The families of 25 females with classical RTT responded and provided written consent for study participation. Data collection included feeding history and assessment of growth using stadiometry. Fasting blood samples were taken for evaluation of blood cell count, lipids, hepatic function, and clotting tests.

All individuals underwent abdominal ultrasonography in order to assess their gallbladder status in supine, left lateral decubitus, and sitting positions. Two examinations were performed: the first was done in a fasting condition (at least 4 hours) and a second scanning was conducted 30 minutes after the ingestion of a standard fatty meal (breakfast). Beside the detection of gallstones or sludge in the intrahepatic biliary tracts, pre- and postprandial gallbladder volume (fasting volume, residual volume, contraction index[15]) and septums or kinking of the gallbladder were evaluated.

Statistical analyses

Body mass index (BMI, kilogram/m^2) was calculated from height and weight measurements. Computations of z scores for height, weight, and BMI were made using the National Center for Health Statistics standards.[21] Data were tabulated using standard descriptive statistics. Two sample Student's *t*-tests were performed to detect differences in fasting volume, residual volume and contraction index between this clinical sample and control females and females with Down syndrome reported by Tasdemir.[15]

Part 3: Assessment and management guidelines

A literature search of electronic databases and online libraries of guidelines was undertaken using keywords of Rett syndrome, developmental disability, intellectual disability, comorbidity, and gallbladder-related terms. The search was limited to full papers in English from 1986 to 2011. Statements relevant to the clinical assessment and management of gallbladder disease in RTT were extracted from the full text.

Clinicians with experience with RTT from different countries in the disciplines of developmental paediatrics and child neurology, gastroenterology, paediatric surgery and clinical genetics were identified through networks of researchers, publications, and the Australian Rett Syndrome[19] and InterRett[20] Databases. Each was invited to participate in a

Delphi study aiming to develop assessment and treatment recommendations for gastrointestinal issues in RTT including poor growth, gastroesophageal reflux, constipation, and abdominal bloating.[22] Of the 20 medical clinicians participating in the larger study, 16 (80.0%) participated in the section on gallbladder disease.

Referenced statements extracted from the literature were accompanied by a 5-point Likert scale for agreement rating (strongly agree, agree, neither agree or disagree, disagree, strongly disagree) with space for comments. The statements and questions were listed in a Microsoft Word document format and an online version was also created as previously described.[22] Consensus was attained when a minimum of 70% of responses were within one response category of the median response.[22] Response to the first round of the Delphi process informed the second set of items until consensus was reached. A level of evidence was applied to each item for which there was consensus: level 1 representing evidence from systematic reviews and randomised controlled trials, level 2 case control or cohort studies, level 3 case reports or case series, and level 4 expert opinion.[23]

The statistical analyses were conducted using Stata version 12 (StataCorp 2012).

Ethical approval for Part 1 of the study was provided by the ethics committee of Princess Margaret Hospital in Western Australia (1909/EP). Part 2 was approved by the ethics committee of the Medical University Vienna. Part 3 was approved by the ethics committee of the University of Western Australia.

RESULTS

Part 1: Occurrence of gallbladder disease

There were 270 individuals from ARSD and 749 from InterRett with a pathogenic *MECP2* mutation who were eligible for the study. The mean observation times were 16.2 years (SD 8.6) and 10.5 years (SD 9.4) for the ARSD and InterRett cohort respectively.

At the time of data analysis, ten individuals from ARSD and 14 from InterRett had been diagnosed with cholelithiasis and/or underwent cholecystectomy. The youngest age of cholecystectomy was 3 years in one of the Australian females, who also had a diagnosis of Down syndrome.[17] The remainder of individuals in the ARSD cohort were 18, 20, 22, 22, 24, 25, 29, 30, and 31 years old. A similar pattern was noted in the InterRett cohort, with four individuals younger than 18 years (4, 12, 15, 17), with the remaining (*n*=10) aged 18 to 47 years. The incidence rate of occurrence of gallbladder disease was 2.3 (95% CI 1.1 to 4.2) and 1.8 (95% CI 1.0 to 3.0) per 1000 person-years in the ARSD and InterRett cohorts

respectively. This equates to approximately two new cases of gallbladder disorder if 100 individuals with RTT are followed for 10 years.

Part 2: Gallbladder function in a clinic sample

The mean age of the 25 females was 16.4 (SD 10.7) years (range 3.4-47.8). A pathogenic mutation was identified in 17 out of 25, and no mutation or one with uncertain pathogenicity in 8 out of 25. Twenty were able to eat varied consistencies of food, three (12%) ate pureed food, and two were fed via a gastrostomy. Caregiver-reported gastrointestinal symptoms including recurrent unexplained vomiting in 3 out of 25 and suspected abdominal pain in 9 out of 25. No history of gallstones or cholecystectomy was reported, although there was a family history in 9 out of 24. The females were usually underweight with a mean BMI z score of -2.8 (SD 3.6, range -13.8 to 1.6). Laboratory data from fasting blood tests revealed slightly elevated bile acids in 9 out of 25 patients and mild hypercholesteraemia in 8 out of 25 patients. Glutamate dehydrogenase, complete blood cell count, electrolytes, liver and renal function tests, and also clotting tests were within normal ranges for age.

The biliary tract was normal in all participants, although the gallbladder was septated in 6 out of 25. There was gallbladder sludge in 1 out of 25 but no gallstones were found. The mean fasting volume was 7755 (SD 7898 mm^3; n=16), and the mean residual volume 3624 (SD 3609 mm^3; n=16). The mean contractility index was 46.5 (SD 38.3%) similar to what has been reported for Down syndrome[15] but lower than that reported for healthy controls (Table II).[15]

Part 3: Assessment and management guidelines

The recommendations of the expert panel of clinicians are shown in Table III. In summary, screaming and apparent abdominal pain were considered suggestive of gallbladder disease, often in association with fever and vomiting.[24,25] After excluding gastrooesophageal reflux as the source of pain,[26] the panel recommended diagnostic tests including ultrasound to examine for gallstones and a hepatobiliary iminodiacetic acid scan to examine the rate at which bile is ejected from the gallbladder.[25,27] There was no consensus in favour of or against use of ursodeoxycholic acid to dissolve gallstones in RTT. In addition to appropriate analgesia, cholecystectomy was recommended for symptomatic biliary dyskinesia, cholelithiasis and in all symptomatic patients with calcified stones,[24,28,29] but not for those with cholecystitis or when asymptomatic (Table III).

DISCUSSION

Our analyses indicate that the prevalence of gallbladder disorder in RTT is approximately 2% in females who are followed for 10 years. This prevalence appears greater than in the general paediatric population but similar to that reported for individuals with Down syndrome. The gallbladder in females with RTT was small with poor contractility compared with healthy controls. Gallbladder disorders should be considered in the differential diagnosis when a female with RTT presents with abdominal pain and clinicians need to be aware of this possibility and its management.

Established in 1993, the Australian Rett Syndrome Database ascertains cases from multiple sources, has collected longitudinal population data,[19] and is thus an ideal research tool to estimate the epidemiology of clinical aspects of RTT. The incidence rate was 2.1 per 1000 person-years in the ARSD cohort, equating to approximately two new cases of gallbladder disorder if 100 individuals are followed for 10 years. Analysis of the InterRett data found a similar incidence rate and, although not population-based, the sample size is large,[20] providing opportunity to confirm the population-based findings. These parallel analyses found that, although gallbladder cholelithiasis and/or cholecystectomy are still rare in RTT, they do occur more commonly than would be expected in females of a similar age in the general population.

Pain is the main symptom of gallbladder disease, usually occurring in intense and recurrent attacks, but it can become less severe and intermittent with time. If cholecystitis develops, there may also be symptoms of fever, jaundice, and vomiting.[26] The communication deficit in RTT creates additional challenges for the assessment of abdominal pain, which can be further complicated by altered pain sensitivity, with decreased sensitivity to pain observed more frequently than increased sensitivity to pain, although increased sensitivity to pain may relate to internal rather than external sources of pain.[18] Visceral pain possesses unique characteristics: both vagal and spinal afferents contribute to the sensory experience, and emotional and autonomic components are also strong.[30] It is not surprising that pain relating to gallbladder disease can be extremely distressing to the female with RTT and also problematic to diagnose. In the presence of abdominal pain, a thorough clinical history and examination taking account of gastrooesophageal reflux and gallbladder disorder should be included in the differential diagnosis. There is no consensus for medical treatment of gallstones with ursodeoxycholic acid in keeping with the poor evidence base for its effectiveness. Rather, if symptomatic, use of cholecystectomy was the treatment of

choice.[24,28,29] Follow-up and clinical review of those with RTT who undergo cholecystectomy is necessary to develop the evidence-base for this painful comorbidity in RTT.

Ingestion of inadequate quantities of food and fluids, low levels of mobility, infections, and medications can all contribute to inadequate gallbladder contraction and emptying, leading to bile stasis, sludge, cholecystitis and development of gallstones.[8,24] Repeated episodes of cholecystitis cause scarring of the gallbladder and further loss of gallbladder motility. The gastrointestinal dysmotility frequently found in RTT may likely be associated with gallbladder disease. We found that the mean contractility index for our clinical sample was 46.5% suggesting gallbladder hypomotility, although there was no evidence of gallstones in any of the 25 individuals. This index was at a similar level to that reported (41.2%) in a small sample of Down syndrome patients (n=9) and markedly lower than reported (75.0%) for healthy control individuals (n=10).[15] In Down syndrome, gallbladder abnormalities have been found in 9.1% (6.9% cholelithiasis and 2.1% biliary sludge),[31] and diagnosed gallstones in 6.9%[9] and 4.7%.[13] Similar to Down syndrome, the immature enteric nervous system in females with RTT might have contributed to the dysfunction in the gastrointestinal tract.[32,33]

The exact pathophysiological cause of the problem has yet to be found,[33] although a role for aberrant *MECP2* gene function in the pathogenesis of gallbladder symptoms is possible. Abnormal cholesterol metabolism has recently been identified in male *Mecp2*-null and female *Mecp2* mutant mice. In comparison with control individuals, these mice had higher lipid concentrations thought to arise from systemic dyslipidaemia, which is also a risk factor for gallstones.[34] Although the underlying mechanisms are unknown, the mouse data support the idea that genes involved in lipid homeostasis are regulated by chromatin-remodelling complexes that involve the *MECP2* gene.[35] Biological processes associated with gallbladder function such as cholesterol metabolism, biliary lipid secretion, lipid transport, and gallbladder contractility are regulated by a variety of transcription factors and nuclear receptors including the liver X receptor (LXR), the farnesoid X receptor (FXR) and the oestrogen receptor alpha (ESR1).[36] The MeCP2 protein is involved in the epigenetic regulation of the oestrogen receptor alpha ESR1,[37,38] and this receptor has been implicated in abnormal cholesterol metabolism leading to gallstone disease.[36] It is thus possible that aberrant *MECP2* gene function contributes to gallbladder disease, through multiple gene regulatory pathways.

Epidemiological estimates were based on population-based and longitudinal data, and any missing data would contribute to a higher estimate. Our prediction of occurrence is

therefore more likely to be an underestimate. Our clinical sample was small but our findings were still statistically significant suggesting that Type II error was unlikely. For our consensus study, the level of evidence was low and of necessity, our expert panel drew heavily on their clinical expertise.

We have conducted the first population-based estimate of the epidemiology of gallbladder disorder in RTT using the Australian Rett Syndrome Database, confirming the findings using the large sample size available in the InterRett. Our ultrasonographic data identified smaller gallbladder volumes and decreased contractility in RTT compared with healthy control individuals and similar to those with Down syndrome. Gallbladder disease appears to occur more commonly in RTT than in the general population and therefore is considered in the differential diagnosis of abdominal pain when it presents in this disorder. The consensus of 16 expert clinicians on the assessment and management of gallbladder disease in RTT represents a platform for the development of more consistent clinical care and research initiatives on this infrequent entity in a rare disorder.

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Table I: Literature review of epidemiology of gallbladder disorders in children.

Reference	Inclusion criteria	Study design and population	Point prevalence of gallbladder disease, % (95% CI)
Koebnick et al 2012.[10]	Cholelithiasis or choledocholithiasis from medical records within 2 years of enrolment	Population-based, members of the Kaiser Permanente Southern California health plan Cross-sectional 510 816 children Mean age 15.1±2.9 years (range 10–19 years)	• 766 patients with gallstones identified (766/510 816, 0.15%) • Point prevalence: Total 0.15 (0.14 to 0.16) Male 0.06 (0.05 to 0.07) Female 0.24 (0.22 to 0.26)
Kratzer et al 2010.[11]	Cholelithiasis on ultrasonography	Population-based, random sampling in an urban area of a small town in Germany Cross-sectional 307 children Mean age 15.1±2 years (range 12-18 years)	• %, showed gallstones (1 female and 2 male adolescents) • Point prevalence: Total 0.98 (- to 2.08) Male 1.33 (- to 3.17) Female 0.64 (- to 1.88)
Toscano et al. 2001.[13]	Cholelithiasis on ultrasonography	Clinical study of patients referred for problems unrelated to gallstones Cross-sectional 139 children with Down syndrome Age: median 36mo, range 1mo-19y 577 healthy controls Age: median 40mo, range 1mo to 19y	• Point prevalence: Total 1.00 (0.26 to 1.73) DS 4.76 (1.04 to 8.48) Control 0.17%(- to 0.51) (No female/male data in those without gallbladder disease hence no prevalence by sex) • 4.7% of those with DS had cholelithiasis, 4 females under 21 months and 2 males aged 6 years (out of 126 patients). One 7-year-old female in the control group was diagnosed with cholelithiasis (0.2%)
Wesdorp et al. 2000[8]	Cholelithiasis	Clinical study 4200 children Age: median ~11y, range 0–18y	• Idiopathic gallstones were found in 19 (23%) patients. Gallstones in association with haemolytic disease in 32 (39%) • Point prevalence (*n*=4200): 1.95 (1.53 to 2.37)

Palasciano et al. 1989[9]	Gallstone presence and gallbladder volume	Non-random sampling from town of Bari, Italy (population 400 000) Cross sectional 1492 children Age: mean 12±4y, range 6–19y	• Overall prevalence of gallstone disease = 0.13%. • Point prevalence: Total 0.13 (- to 0.32) Male 0 Female 0.27 (- to 0.64)
Nomura et al. 1988[12]	Presence of gallstones on ultrasound	Population-based study, >83% of population in the Yaeyama District of Okinawa 742 children Age: 0–19y 239 adults Age: 20–29y 292 adults Age: 30–39y	• 0% in under 19 years of age 20–29 year olds ~3.0% 30 -39 year olds ~3.5% • Point prevalence in the 20–39y age group: Total 2.07 (0.86 to 3.28) Male 1.38 (0.04 to 2.73) Female 2.89 (0.78 to 5.00)
Everhart et al. 1999[39]	Presence of cholelithiasis	Population-based as part of the NHANES III study Cross-sectional 14 238 adults 3420 were 20–29y 3231 were 30–39y	• Point prevalence in 20–29y: Total 101/3420 = 2.95 (2.39 to 3.52) Male 21/1602 = 1.31 (0.75 to 1.87) Female 80/1818 = 4.40 (3.46 to 5.34) • Point prevalence in 30–39y: Total 110/3231 = 3.40 (2.78 to 4.03) Male 16/1426 = 1.12 (0.58 to 1.67) Female 94/1805 = 5.21 (3.46 to 5.34)
Tyler et al. 2004[14]	Presence of gallbladder disease including cholelithiasis	Clinical case control study Down syndrome, $n = 28$ Age: mean 43.0±10.0y Healthy controls, $n = 112$	• Prevalence for total sample: Total 8.57 (3.93 to 13.21) Male 3.08 (- to 7.28) Female 13.33 (5.64 to 21.03)

		Age: mean 43.1±10.0y	- Prevalence for those with Down syndrome: 25.00 (8.96 to 41.04) Male 7.69 (- to 22.18) Female 40.00 (15.21 to 64.79) - Prevalence for control children Control 4.46 (0.64 to 8.29) Male 1.92 (- to 5.66) Female 6.67 (0.35 to 12.98)

Table II: Gallbladder fasting volume, resting volume and contraction indices for females with Rett syndrome, Down syndrome[a] and in healthy control individuals.[a]

	Rett syndrome (*n*=25) Mean (SD)	Down syndrome (*n*=9) Mean (SD)	Healthy controls (*n*=10) Mean (SD)	Mean difference: Rett and Down syndrome — Mean difference (95% CI)	*p*	Mean difference: Rett syndrome and healthy — Mean difference (95% CI)	*p*
Fasting volume (mm^3)	7755.2 (7898.9)	5996.4 (3388.2)	13 821.2 (5811.4)	1811.5 (-1935.1 to 5558.0)	0.331	-6,013.3 (-10 879 to -1147.7)	0.018
Residual volume (mm^3)	3624.8 (3609.9)[b]	3123.4 (1608.7)	5026.9 (1202.4)	501.4 (-1674.2 to 2677.0)	0.638	-1402.1 (-3446.5 to 642.3)	0.168
Contraction index (%)	46.5 (38.27)[b]	44.2 (17.6)	79.2 (13.6)	2.3 (-20.9 to 25.6)	0.839	-32.7 (-54.6 to -10.8)	0.005

[a] Group data describing individuals with Down syndrome and healthy control individuals derived from Tasdemir (2004).[15]

[b] *n*=16.

Table III: Clinical assessment and treatment of gallbladder problems in Rett syndrome.

Item	Level of evidence[a]	Median response	n/N [b] (%)
Assessment			
1. Screaming or apparent abdominal pain is suggestive of gallbladder disease[24]	4	Agree	15/16 (93.8)
2. The triad of apparent pain, vomiting and fever is the usual mode of presentation of cholecystitis[25]	3	Agree	16/16 (100)
3. Exclude GERD as a cause of pain[26]	3	Agree	15/16 (93.8)
4. An ultrasound scan can be used to identify the presence of gallstones[25]	3	Agree	15/16 (93.8)
5. Oral cholecystogram or a CCK or HIDA scan can be used to confirm biliary dyskinesia[25,27,29]	3, 3, 3	Agree	13/13 (100)
Treatment			
1. Ursodeoxycholic acid may be considered in an asymptomatic patient with gallstone(s)		Neither agree or disagree	10/11 (90.9)
2. The treatment of cholecystitis is cholecystectomy		Neither agree or disagree	13/14 (92.9)
3. Cholecystectomy can be considered in cases of cholecystitis after antibiotic treatment		Agree	14/15 (93.3)
4. Cholecystectomy is advised for all non-symptomatic patients with sludge or non-calcified stones that have not resolved in 2 to 3 months[40]	3	Neither agree or disagree	10/12 (83.3)
4. The treatment of symptomatic biliary dyskinesia is cholecystectomy[28]	3	Agree	11/13 (84.6)
5. The treatment of cholelithiasis is cholecystectomy[24]	4	Agree	13/14 (92.9)

GERD, Gastro-esophageal reflux disease; CCK, Cholecystokinin;HIDA, hepatobiliary iminodiacetic acid.

[a] Scottish Intercollegiate Guidelines Network.

[b] numerator is the number of responses with median response or one category either side and denominator is the number of clinicians in the panel whose expertise was relevant to this item.

Appendix S1

Members of the expert panel participating in Delphi technique in relation to gallbladder disease in Rett syndrome