Healthy volunteers (HV). Each participant underwent two ten-minute gradient echo-planar imaging scans, one directly after the other. FSL (Oxford, UK) was used for data pre-processing, brain extraction and registration of functional data into standard space. Single subject independent component analysis (ICA), followed by a group ICA and dual regression were used to identify differences in resting-state network activity between HV and RUCC using FSL. Due to processing limitations, both scans for each subject were entered into the analysis as an individual data set, which may have inflated significance. This is a preliminary analysis, with findings to be confirmed in the full study cohort (50 RUCC vs 20 HV).

**Results** Age, lung function and BMI were comparable between groups. RUCC median (IQR) age was 66 (60–72), 9/13 were female and mean duration of cough was 11 years. Median (IQR) day VAS 46mm (28–68), CQLQ 59 (42–70) and day cough frequency 47 coughs/hr (15–69). There were significant differences in CNS activity between RUCC and HV groups in the cingulate gyrus and sensorimotor areas of the brain (p<0.05). These differences are still present when p<0.005. **Figure 1** Independent components identified by a group analysis comprising of sensorimotor network areas (light grey) and between group differences in activity (RUCC vs HV, p<0.05, dark grey).

**Conclusions** Functional alterations in CNS activity may contribute to the pathophysiology of RUCC.

**P215 RFC1 DISORDER; A GENETIC, NEUROPATHIC CAUSE OF CHRONIC COUGH**

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**Introduction** Chronic cough (CC, lasting >8 weeks) is often the presenting symptom of CANVAS; Cerebellar Ataxia, Neuropathy, and Vestibular Areflexia Syndrome. CANVAS is a late onset, autosomal recessive, neurodegenerative condition caused by biallelic repeat expansions in the RFC1 gene (RFC1++) and with no RFC1 repeat expansions (RFC1–).
by biallelic repeat expansions in the RFC1 gene (RE-RFC1). We investigated the prevalence of biallelic AAGGG repeat expansions in RFC1 and clinical characteristics of patients with CC.

**Methods** Consecutive patients attending a specialist CC clinic underwent RFC1 genetic testing by polymerase chain reaction (PCR). CC was managed as per ERS guideline. Cough symptoms were assessed with cough severity VAS and health status (LCQ). Objective 24hr cough frequency (CF) was measured with Leicester Cough Monitor. Patients were screened for CANVAS neurological symptoms.

**Results** Patients with CC underwent RFC1 testing; n=51, female 71%, median (IQR) age 65 (56–70) years, duration of cough 155 (80–240) months, and 50 (98%) had refractory or unexplained cough. Patients had median (IQR) cough severity VAS 68 (51–76), LCQ 9.7 (7.9–12.3), CF geometric mean (logSD) 19.4 (1.7) coughs/hr. Four patients (8%) had biallelic AAGGG RE-RFC1 (RFC1++) (female 75%, median (IQR) age 69 (59–74)). Monoallelic AAGGG RE-RFC1 (carrier) was present in 5 (10)%. Compared to patients with no RFC1 repeat expansions (RFC1−, n=42), RFC1++ had a longer duration of cough and higher severity VAS; median (IQR) 258 (198–336) vs. 149 (71–240) months, and 77 (70–81) vs. 66 (50–73), respectively. There was no significant difference between RFC1++ and RFC1− in age, sex, BMI, spirometry, LCQ, or CF. Self-reported neurological symptoms were common in patients with CC irrespective of RFC1 status (figure 1); however, pins and needles were reported in all RFC1++ compared to 39% in RFC1−.

**Discussion** RFC1 disorder was the cause of unexplained or refractory chronic cough in 8% of patients attending a specialist clinic. Symptoms suggestive of neuropathy and ataxia were common in both RFC1++ and RFC1− in age, sex, BMI, spirometry, LCQ, or CF. Self-reported neurological symptoms were common in patients with CC irrespective of RFC1 status (figure 1); however, pins and needles were reported in all RFC1++ compared to 39% in RFC1−.

**Conclusion** Whole blood ATP was higher in RUCC compared to HV, but this was not replicated in plasma samples. It is unclear whether the different assays or different sample types account for this discrepancy. It is unknown whether whole blood ATP is a good surrogate for extracellular ATP levels. Between assay differences should be considered when interpreting ATP results.

**P216 WHOLE BLOOD AND PLASMA ATP LEVELS IN REFRACTORY/UNEXPLAINED CHRONIC COUGH**

**Introduction** The efficacy of P2X3 antagonists in refractory/unexplained chronic cough (RUCC) suggests a role for extracellular ATP in the airways. We aimed to compare whole blood ATP and plasma ATP between RUCC and healthy volunteers (HV) and evaluate if ATP measurements correlate with other measures of cough severity.

**Methods** Whole blood was collected in two EDTA tubes (EDTA 1.6 mg/ml) from 50 patients with RUCC and 20 HVs, then diluted (× 10,000) in PBS. ATP was measured (SystemSure bioluminescence assay) twice, and a third time if discordance >20% between 1st and 2nd samples. A second EDTA sample was centrifuged (1300 xg, 10min, 4°C), and plasma transferred to 2 ml cryotubes and frozen (-80°C). ATP was measured in plasma samples using the ATP Detection assay kit (Cayman).

**Results** Age, BMI, smoking status, and lung function were comparable between groups. SystemSure assay reliability was 0.79 (Cronbach Alpha). Median (IQR) all samples (RUCC n=126/HV n=48) whole blood RLU was higher in RUCC 346 (275–490) than HV 283 (204–489), (p=0.01), but was not significant in 1st sample (p=0.22) or mean sample (p=0.18). By contrast median relative light units (RLU) assessed by plate-based luminescence assay was not different in plasma between RUCC 21050 (14741–27155) and HV 19515 (14109–30948). The only significant correlation between measures (mean whole blood RLU, plasma RLU) and variables (age, FVC predicted or daytime cough frequency) was a weak inverse relationship between plasma RLU and age (r=–0.243, p=0.043; Spearman’s).

**Conclusion** Whole blood ATP was higher in RUCC compared to HV, but this was not replicated in plasma samples. It is unclear whether the different assays or different sample types account for this discrepancy. It is unknown whether whole blood ATP is a good surrogate for extracellular ATP levels. Between assay differences should be considered when interpreting ATP results.

**P217 NIGHT-TIME COUGH FREQUENCY: RELATIONSHIP WITH AWAKE COUGH FREQUENCY**

**Introduction** Objective ambulatory cough frequency (CF) monitoring is usually assessed over 24 hours. This is inconvenient for some patients and its use has therefore largely been restricted to the research setting. Night-time only objective CF monitoring could reduce ambient noise, and reduce the burden on patients compared to 24-hour CF monitoring. This study aimed to investigate the relationship of night-time CF with awake CF, as well as it’s impact on sleep disturbance.

**Methods** A prospective study of consecutive patients with refractory or unexplained chronic cough completed ambulatory 24-hour cough monitoring with the Leicester Cough Monitor (LCM). Participants completed a diary to report sleep and awake times. Night-time and awake CF were measured. Participants completed cough severity visual analogue scale (VAS) and cough-specific health status Leicester Cough Questionnaire (LCQ). Question 10 of the LCQ was used to assess sleep disturbance (‘In the last 2 weeks, has your cough disturbed your sleep?’), which is scored 1–7, with a lower score indicating higher sleep disturbance.

**Results** 44 participants completed 24-hour CF monitoring; mean (SD) age 62 (11) years, (n) 68% female, median (IQR) cough duration 90 (38–225) months, cough severity VAS 70 (55–79) mm and LCQ score 10.8 (8.5–14.1). Geometric mean (SD) night-time CF and awake CF were 5.0 (3.6) and 23.3 (2.1) coughs/hr respectively. Night-time CF was significantly associated with awake CF (r=0.46), 24-hour CF (r=0.58), LCQ score (r=−0.38) and sleep disturbance (r=−0.47) and was higher females vs males (geometric mean (SD)