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Hyposmia, symptoms of REM sleep behavior disorder and Parkinsonian motor signs suggests prodromal neurodegeneration in 22q11 deletion syndrome

Running heads: Prodromal signs in 22q11 deletion syndrome.

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ABSTRACT

Objective: The 22q11 deletion syndrome is one of the most common genomic disorders in man. There is an increased risk of Parkinson's disease in people with 22q11 deletion syndrome. The characteristic motor features of Parkinson's disease begin when more than 50% of dopaminergic neurons in the substantia nigra have degenerated. Prior to this there is a prodromal period, of up to 20 years, in which non-motor features such as hyposmia, autonomic dysfunction, REM sleep behavior disorder and subtle motor dysfunction can occur. Methods: We used validated clinical tools to investigate the presence of prodromal markers of Parkinsons disease in 50 adults with 22q11 deletion syndrome and 143 matched deletion negative controls. Results: The median score on the University of Pennsylvania Smell Identification Test was significantly lower in the 22q11 deletion group, and 44% scored in the hyposmic range (p=0.024). Individuals with 22q11 deletion syndrome were significantly more likely to report automomic symptoms (urinary dysfunction or constipation, p=0.016). Twenty-eight percent of 22q11 deletion syndrome participants scored above the threshold for REM sleep behavior disorder on a screening questionnaire (p=0.022). Four 22q11 deletion syndrome participants had Parkinsonian motor signs on examination, which did not meet diagnostic criteria for Parkinson's disease. Conclusion: We report prodromal markers of Parkinson's disease in 22q11 deletion syndrome. These may help identify people with 22q11 deletion at risk of neurological

disease. However, the significance of these signs needs to be confirmed by longitudinal studies of development of Parkinson's disease.

Key words: Parkinson's disease, movement disorder, 22q11 deletion syndrome, hyposmia, REM sleep behavior disorder.

INTRODUCTION

The 22q11 deletion syndrome (22q11DS) (OMIM 611867) is caused by deletion of a 1.5 – 3 Mb segment of the long arm of chromosome 22 at band 11 [1]. It is one of the most common genomic disorders in man, affecting around 1/2 -3 000 people. The majority of people with 22q11DS have mild to moderate intellectual disability [1]. Other frequent features of 22q11DS include congenital heart disease, cleft lip or palate, thyroid dysfunction and hypoparathyroidism with associated hypocalcaemia [1].

Recent studies have indicated an association between 22q11DS and Parkinson's disease (PD) [2, 3]. In a cohort of 159 adults with 22q11DS a standardized morbidity ratio for PD of 69.7 was reported [2]. The age of motor symptom onset was 39-48 years. In a study of over 9 000 cases of PD, 8 were found to carry a 22q11 deletion with a median age of onset of PD symptoms of 37 years [3].

The classic motor triad of PD (bradykinesia, rest tremor and postural instability) develops once more than 50% of dopaminergic neurons in the *substantia nigra* have degenerated [4]. Preceding this there is a long prodromal period in which it is non-motor features of PD that predominate [4]. This prodromal period is proposed to last up to 20 years [4]. Non-motor features that occur in this period are

termed *prodromal markers of neurodegeneration*. Prodromal markers include hyposmia [5], autonomic dysfunction [6], REM sleep behavior disorder [7], and subtle motor impairment [8]. These can be assessed using a range of validated clinical rating scales. Here we report a multicenter, observational study of the presence of prodromal markers of neurodegeneration in adults with 22q11DS.

METHODS

22q11DS participants and controls

Individuals over the age of 18 years old with a 22q11 deletion were recruited from Regional Clinical Genetics Centers across the United Kingdom (UK) through the National Institute of Healthcare Research (NIHR) "Musketeers memorandum". This memorandum permits nationwide recruitment of participants for a rare disease study run by a single center. Ethical approval was granted by South West - Central Bristol Research Ethics Committee (15/SW/0272). Eligible participants had a 22q11 deletion identified by a standard clinical diagnostic technique (Karyotype, fluorescent in situ hybridization or comparative genomic hybridization). Age and sex matched controls were recruited from parents or siblings who were negative for the 22q11 deletion. All participants gave written informed consent.

Clinical evaluation

All procedures were performed identically in 22q11DS participants and controls by a Consultant Clinical Geneticist with a special interest in Neurogenetics (AM). The clinical features of 22q11DS in 22q11DS participants were assessed with a structured medical interview (drug history included current use of anti-depressant medication and both current and previous use of anti-psychotics) and the Sinonasal outcome test (SNOT-22) for upper airways symptoms [9]. Participants were

evaluated using the Movement Disorders Society Unified Parkinson's Disease Rating Scale activities of daily living and motor subscale (UPDRS parts I, II and III)[10]. Strict criteria were applied for the definition of PD: at least 2 of asymmetry, bradykinetic-rigid syndrome, and resting tremor, with excellent response to dopaminergic therapy (if treated).

Odor identification was assessed with the 40-item University of Pennsylvania Smell Identification Test (UPSIT - Sensonics Inc, Haddon Heights, New Jersey, USA), which has been used in several published UK cohorts. Hyposmia was defined using age and sex adjusted centiles. Individuals with anatomical lesions of their upper airways, upper respiratory infections, or who were smokers were excluded. Cognitive function was assessed with the Montreal Cognitive Assessment (MoCA). The MoCA is more sensitive in detecting cognitive deficits in PD compared to the MMSE[11], with a score of <26 signifying mild cognitive impairment and <24 dementia [11]. Features of RBD were screened for with the RBD Questionnaire (using a cut off of 5 or more as indicting possible RBD [12]) and daytime sleepiness was assessed with the Epworth sleepiness scale (ESS)[13]. Depression was screened for with the Beck Depression Inventory (BDI), using a cut off score of 10 for depression [14]. Symptoms of autonomic dysfunction (urinary, constipation, postural symptoms) were assessed using UPDRS part I. The presence or absence of an autonomic symptom was summed to give a score ~~out~~ of 0-3 for each participant.

Statistical analysis

All analysis was performed using SPSS (version 23, IBM computing). Raw UPSIT scores, MoCA, RBD, SNOT-22, BDI and UPDRS I, II and III scores are not normally distributed. Differences between groups ~~medians~~ were assessed using the

Mann-Whitney U-test. Student *t* test and the chi squared test were used to check differences in age and sex between groups. ~~Correlations between variables were assessed with a bivariate analysis using Spearman's correlation.~~ Significance was defined at the 5% level.

RESULTS

Baseline characteristics of 22q11DS cohort

Fifty individuals with 22q11DS were recruited (19/50 male, mean age 32 years +/- standard deviation of 11 years, range 18-57) along with 1~~4~~3 matched healthy controls (4/1~~4~~3 male, mean age 39~~4~~0 years +/- standard deviation of 19 years, range 18-70). Neither age (students t-test. t=1.74 p=0.0~~8~~6) nor sex (chi squared p=0.7) differed between cases and controls. All cases and controls were of white British ethnicity. None of the cases or controls ~~smoked or~~ drank alcohol beyond recommended limits or smoked. All 22q11DS participants had typical features of the condition (table 1). All 22q11DS participants had apparent mild intellectual disability (defined as requiring additional help in a mainstream school). As expected, the median SNOT-22 score was significantly higher in 22q11DS participants than controls (median 2 [IQR (interquartile range) 0-17] vs median 0 [IQR -0-0] p=0.003).

Potential clinical markers of prodromal neurodegeneration in 22q11DS

Figure 1 summarizes median scores for non-motor prodromal markers. The median UPSIT score was significantly lower in 22q11DS than matched controls (median 27 [IQR 22-29] vs median 34 [IQR 31-36], U=44, Z = -4.9, p<0.01) and significantly more 22q11DS participants scored in the hyposmic range (22/50 [44%] vs 0/13, Fischers exact test, p=0.0024). ~~The UPSIT score did not correlate with the~~

~~SNOT-22 score (Spearman's rho -.1, p=0.46).~~ The RBD sleep disorder questionnaire score was significantly higher in 22q11 DS (median 3 [IQR 1-6] vs median 0 [IQR 0-4], U=163, Z= -3.0p=0.004). Significantly more participants with 22q11DS scored above the threshold for REM sleep disorder on the screening questionnaire (14/50 [28%] vs 0/13, chi squared p = 0.022). There was no significant difference for the Epworth sleepiness score (median 4 [IQR 0-9] vs median 0 [IQR 0-7], U=282, Z=-1.1, p=0.29). As expected, the median score on the MoCA was significantly lower in 22q11DS (median 22 [IQR21 -26] vs median 29 [IQR 28-30]), U=37, Z=-5.0, p<0.01). Both the UPDRS part 1 (median 4 [IQR 1 – 7] vs median 0 [IQR 0 – 0], U=83, Z=-4.4, p<0.01) and UPDRS part 2 (median 2 (IQR 0-3) vs median 0 [IQR0-0], U=140, Z=-3.7, p<0.01) scores were significantly higher in 22q11DS. The median BDI score was significantly higher in 22q11DS (median 1 [IQR 0-7] vs median 0 [IQR 0-0.5], U=226, Z=-2.0, p=0.04). Autonomic symptoms were more common in the 22q11 group (median 0 [IQR 0-1] vs median 0 [IQR 0-0], U=272, Z=-2.5, p=0.016).

Parkinsonian motor signs in adults with 22q11DS

The UPDRS part III score was significantly higher in 22q11DS than controls (median 1 [IQR 0-6] vs median 0 [IQR 0-0], U=158, Z=-3.3, p=0.01). Four 22q11DS participants had motor features, which were distinct from normal but did not meet diagnostic criteria for motor Parkinsonism. DGS1 had right sided rigidity with activation maneuver, slight decrementing of amplitude of finger tapping of the right hand and right sided postural tremor. DGS30 had rigidity of the right arm with activation maneuver, and slow and irregular finger tapping with reduced arm swing when walking. DGS46 manifested bilateral upper limb rigidity with activation maneuver, hunched posture and reduced left arm swing when walking. DGS47 displayed masked facies (reduced blinking and few spontaneous lip movements),

decrementing amplitude of hand opening-closing with several freezing episodes, and slowness of gait. None of these 4 individuals had used anti-psychotic medication.

No participant met diagnostic criteria for PD. However, participant DGS02 and DGS11 had generalized myoclonus, DGS06 had facial motor tics and DGS12 had nocturnal restless legs. DGS17 had minimal masked facies, upper limb rigidity with activation maneuver, mild unilateral slowing of finger tapping and bilateral reduced arm swing when walking associated with anti-psychotic use. Even wWith these 9 individuals excluded the median UPDRS part III score remained higher in the 22q11DS group (median 1 [IQR 0-5], U=158, Z=-2.8, P=0.01).

~~Correlations~~ Co-occurrence of~~between~~ prodromal markers in 22q11DS

Given that hyposmia, REM sleep behavior disorder and abnormal motor findings are reported to be the prodromal markers with greatest predictive power we examined for co-occurrence of these, Several individuals manifested multiple motor and non-motor prodromal markers. One participant had hyposmia, abnormal motor examination and scored above the cut-off for REM sleep behavior disorder. Five participants had hyposmia and scored above the cut-off for REM sleep behavior disorder. Two participants had hyposmia and an abnormal motor examination.

DISCUSSION

Here we describe the presence of clinical features of potential prodromal neurodegeneration in a cohort of adults with 22q11DS. The clinical characteristics of our cohort were similar to those previously reported for adults with 22q11DS [15], but only 8% of our cohort had schizophrenia which is lower than generally reported. To the best of our knowledge, no similar studies have been reported. At the group level,

participants with 22q11DS had impaired olfaction, symptoms of REM sleep behavior disorder and had subtle motor signs. These may represent the earliest phases of a neurodegenerative condition such as PD.

Hyposmia is a widely accepted marker of prodromal neurodegeneration in PD [5]. However, in the general population, only a minority of hyposmic individuals develop PD. We found that 44% of our cohort scored in the hyposmic range on the SIT. Given the high prevalence of hyposmia in our cohort, not all hyposmic individuals can be in the prodrome of a neurodegenerative disorder, and other factors must be involved. However, we did not observe a correlation between SIT and SNOT-22 scores, suggesting that upper airways disease or the sequelae of cleft palate are not the major determinants of olfactory function. This is in keeping with a study of children with 22q11DS, which identified that 68% had hyposmia, and concluded that velopharyngeal insufficiency was not a major causal factor [16]. We excluded smokers and individuals with upper respiratory tract infections to minimize these confounding variables. The pathophysiological explanation for hyposmia in our cohort remains unclear, but it seems likely that hyposmia in 22q11DS is due to prodromal neurodegeneration in only a minority.

Symptoms of REM sleep behavior disorder occurred more frequently in participants with 22q11DS than controls. REM sleep behavior disorder is a strong prodromal marker of neurodegeneration, being highly predictive of development of dementia or a Parkinsonian disorder [17]. However, 22q11DS is associated with obstructive sleep apnea [18]. It is possible that this could mimic symptoms of REM sleep behavior disorder. We contend that it is unlikely that this explains the association between 22q11DS and symptoms of REM sleep behavior disorder, since ~~there was no correlation between RBD questionnaire score and symptoms of upper~~

~~airway obstruction on the SNOT-22. In addition~~, the REM sleep behavior disorder questionnaire is both sensitive and specific for symptoms of REM sleep behavior disorder [12]. If REM sleep behavior disorder in 22q11DS is confirmed by formal sleep studies then it could play an important role in identifying prodromal neurodegeneration in this cohort.

Autonomic symptoms were significantly more common in people with 22q11DS, with 16/50 participants (32%) complaining of constipation and/or urinary dysfunction. Constipation is a well-accepted marker of increased PD risk, with increasing risk of developing PD correlating with decreasing frequency of bowel motions, and may begin 10-20 years before motor presentation [4].

Motor signs such as rigidity or bradykinesia, which do not meet diagnostic criteria for PD, occur in 30-40% of community dwelling older adults [19]. These are termed “mild parkinsonian signs”, and may be a precursor of PD in a subset of individuals. In our cohort, four individuals displayed motor signs that were possibly parkinsonian in nature, but that did not meet diagnostic criteria for PD. These 22q11DS participants had upper limb rigidity with activation maneuver; this can robustly distinguish PD subjects from controls [20]. These individuals also had asymmetrical/reduced arm swing when walking, which is proposed as a prodromal marker of PD [21]. We do not suggest that all individuals with 22q11DS and motor signs will develop PD, but that those with combinations of motor signs and prodromal markers such as hyposmia will be at greatest risk.

It is instructive to compare the results of the current study with other investigations of the PD prodrome. Both individuals with Gaucher disease, and heterozygous carriers of *GBA* mutations, are at increased risk of PD, and these

groups have been shown to exhibit hyposmia and motor signs of subclinical Parkinsonism [22]. Individuals with *LRRK2* mutations, who have not developed motor PD, have subtle motor signs, such as reduced arm swing when walking [21], and hyposmia [23]. The PREDICT-PD study is investigating over 1,000 adults aged 60-80 years for prodromal markers of PD [24]. In this cohort, individuals classified as being at higher risk of PD using epidemiological criteria had an increased prevalence of prodromal markers: 31% were hyposmic and 24% scored over the cut-off for REM sleep disorder [24]. The spectrum and prevalence of prodromal markers reported in other groups at increased risk of PD is similar to what we describe in 22q11DS. This provides a degree of cross-study validation for our findings.

The mechanism by which 22q11DS might predispose to PD is unknown. A recent imaging study suggests increased dopaminergic signaling in 22q11DS [25], which might be neurotoxic and predispose to PD. Here we demonstrate that adults with 22q11DS manifest clinical markers of potential prodromal PD. Longitudinal studies will be required to identify conversion to PD, and validate the clinical significances of these prodromal markers.

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Figure 1. Non-motor potential prodromal markers.

Box plots demonstrate median (heavy horizontal line), 1st to 3rd quartiles (box) and range (whiskers). The outliers (clear circles) were defined automatically by SPSS.

A. The median Smell Identification Test score was significantly lower in 22q11 deletion participants than in controls. B. The median Montreal Cognitive Assessment score was significantly lower in 22q11 deletion participants. C. The median REM sleep behavior disorder questionnaire score was significantly higher in 22q11 deletion participants. D. The median Beck Depression Inventory score was significantly higher in 22q11 deletion participants.

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