INTRODUCTION

Due to the continuing rise in autism spectrum disorder (ASD) prevalence (1 in 88 US children (1)) there is an increasing need to identify the mechanisms underpinning ASD and its diagnosis and treatment. Whilst providing clues about etiology, biomarkers for ASDs could also be used for diagnosis, subtyping, treatment monitoring and identifying therapeutic targets. ASDs are not always detected, especially in younger children, and diagnostic tools available for older children may not be appropriate to use in studies to evaluate therapies in younger children. Sample collection from younger children is not only more difficult but also associated with discomfort. Therefore, a means for detecting ASD in younger children would be highly beneficial.

We analysed the saliva of three 5-17yr old Diagnostic and Statistical Manual of Mental Disorders (DSM-5) ASD children who had been referred to the Regional Developmental Neurology service at Great Ormond Street Hospital for Children, London, United Kingdom for evaluation of potential non-invasive diagnostic biomarkers. The saliva proteome was subject to multidisciplinary analysis to determine the presence of proteins that may be involved in the pathogenesis of ASD.

RESULTS

The saliva of control children exhibited higher levels of Tubulin alpha 1C chain, submaxillary gland androgen regulated protein alpha 1C chain, submaxillary gland androgen regulated protein alpha 1C chain, and Annexin v (Annexin v). The saliva of ASD children showed increased levels of beta 2 microglobulin, heat shock protein alpha B crystallin and beta 2 microglobulin. The differential expression of these proteins suggests that they may be potential biomarkers for ASD.

METHODS

We analysed the saliva of three DSM-5 ASD patients and Statistical Manual of Mental Disorders (DSM-5) ASD diagnosed children and their non-autistic control siblings. Saliva was collected following a strict diet, timing and sampling protocol. The saliva was centrifuged at 13,000 rpm for 10 minutes to remove particulate matter and concentrated to 200 µg of protein. The concentration was assessed using a Nanodrop 1000. The internal standard protein alcohol dehydrogenase from yeast was added to the saliva samples at a ratio of 1:1. The saliva samples were then subjected to trypsin digestion. Reduction, alkylation and digestion were performed as in Martinez et al. (5). 40 µl of each sample, corresponding to 50 µg of protein, was mixed in with 15 µl of 5% (v/v) trypsin in 0.1% (v/v) formic acid and 5% (v/v) acetonitrile solution. The samples were then incubated at 37°C for 1 hour and then for a further 12 hours at 4°C in a 96 well plate. The resulting peptides were then desalted on a C18 column (Agilent Technologies, Santa Clara, CA) and lyophilized. The desalted samples were then reconstituted in 50 µl of 0.1% (v/v) formic acid and 0.1% (v/v) acetonitrile solution. 5 µl of the samples was injected into the HDMSE system for analysis.

The data from the HDMSE system was processed using PLGS software (version 2.0) from Proteome Discoverer 2.0 software (version 2.0). Proteins were identified using the UNIPROT human database. The identification criteria for the proteins were as follows: quantitative characterization of the identified proteins (5).

Regulated proteins provide new avenues of research for a non-invasive saliva based diagnostic for ASD.

CONCLUSION

The saliva of control children exhibited higher levels of Tubulin alpha 1C chain, submaxillary gland androgen regulated protein and Tubulin alpha 1C chain. The saliva of ASD children showed increased levels of beta 2 microglobulin, heat shock protein alpha B crystallin and beta 2 microglobulin. The differential expression of these proteins suggests that they may be potential biomarkers for ASD.

References