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Negative results

Cheek cell–derived α-synuclein and DJ-1 do not differentiate Parkinson's disease from control

Tessandra Stewart [a], Yu-Ting Sui [a], Luis F. Gonzalez-Cuyar [a], David T.W. Wong [b], David M. Akin [b], Vitor Tumas [g], Jan Aasly [h], Emily Ashmore [d], Patrick Aro [a], Carmen Ginghina [a], Ane Korff [a], Cyrus P. Zabetian [c,d], James B. Leverenz [c,e,f], Min Shi [a], Jing Zhang [a,*]

[a] Department of Pathology, University of Washington School of Medicine, Seattle, WA, USA
[b] School of Dentistry, University of California, Los Angeles, CA, USA
[c] Department of Neurology, University of Washington School of Medicine, Seattle, WA, USA
[d] Geriatric Research, Education and Clinical Centre, Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA
[e] Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, WA, USA
[f] Mental Illness Research, Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA
[g] Department of Neuroscience and Behavior, Ribeirão Preto School of Medicine, University of São Paulo, São Paulo, Brazil
[h] Department of Neurology, St Olav's University Hospital, Trondheim, Norway

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ABSTRACT

Recently, α-synuclein (α-syn) and DJ-1, 2 proteins critically involved in Parkinson's disease (PD), have been shown to be present in saliva, suggesting their potential utility as biomarkers of PD. However, the origin and influence of demographic characteristics (e.g., age or sex) on these proteins are unknown. We identified cheek epithelium, which forms the majority of the cellular component of saliva and is readily accessible clinically, as 1 of several potential sources of salivary α-syn and DJ-1. However, no PD-related trend in the cellular component was present. In the supernatant collected from 198 healthy subjects, no correlation was seen between salivary DJ-1 or α-syn with age. When male and female subjects were analyzed separately, a weak age-dependent increase in DJ-1 level was present in male subjects, along with slightly increased α-syn in female subjects. These results, albeit largely negative, provide critical information for understanding the salivary gland pathology and saliva as a PD biomarker source, and must be considered in future investigations of salivary changes in PD.

1. Introduction

Development of therapeutic treatments for Parkinson's disease (PD) is hindered by the lack of robust, noninvasive biomarkers, especially for early disease stages. Recent work demonstrating Lewy body pathology in the salivary glands (Beach et al., 2010; Cersosimo et al., 2010; Del Tredici et al., 2010) suggests that saliva may yield readily accessible biomarkers for PD. We previously identified 2 PD-related proteins, DJ-1 and α-synuclein (α-syn), in human saliva (Devic et al., 2011). Furthermore, we observed a trend toward increasing DJ-1 and decreasing α-syn in PD patients compared to controls, supporting the potential for identification of salivary biomarkers for PD. To characterize the variables contributing to salivary distribution of DJ-1 and α-syn in saliva, this study investigated potential sites of their origin, tested whether their levels in the cellular saliva component distinguish between control and PD subjects, and examined demographic effects on their concentration in a cohort of healthy subjects.

2. Methods

Immunohistochemical staining was performed to visualize DJ-1 and α-syn, along with neurofilament, in cheek and submandibular glands obtained at autopsies of 3 subjects without neurological diseases. Whole saliva was collected from healthy subjects across a wide age range, as well as from PD patients and age-matched controls, as previously described (Devic et al., 2011). DJ-1 and α-syn were measured in lysate obtained from the cellular component of saliva from PD patients and controls as well as in the acellular component of saliva from healthy subjects. Analyses were performed using PASW Statistics 18.0 (SPSS, Inc, Chicago, IL).

* Corresponding author at: Division of Neuropathology, Harborview Medical Center, University of Washington School of Medicine, Box 359635, 325 9th Avenue, Seattle, WA 98104-2499, USA. Tel.: +1 206 897 5245; fax: +1 206 897 5249.
E-mail address: zhangj@u.washington.edu (J. Zhang).

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Nonparametric tests (Mann-Whitney) were used to analyze differences in the distribution by sex of DJ-1 and α-syn in saliva supernatants and pellets. Linear regression was used to examine the relationships between age and analytes. Power analysis was carried out using R v. 2.15.2. Supplemental Methods in Appendix A provide further details.

3. Results

DJ-1 was clearly present in cheek squamous epithelium (Fig. 1A), as well as underlying muscle, nerve, and vascular structures (Fig. 1G). In contrast, the basal cheek epithelial layer stained for α-syn (Fig. 1B), but superficial layers and underlying tissue did not (not shown). We further examined the distribution of both proteins in submandibular glands of subjects without neurodegenerative disease. We observed DJ-1 staining throughout the gland and innervating nerve fibers (Fig. 1F), and α-syn in the nerve but not the gland (Fig. 1C, E), similar to the distribution of neurofilament (Fig. 1D).

DJ-1 and α-syn are detectable in both cellular (pellet) and acellular (supernatant) components of saliva. Because multiple potential sources may contribute to DJ-1 and α-syn in saliva, we chose to determine whether the most proximal (i.e., within the mouth) and clinically accessible source, the cheek epithelial cells, can differentiate between PD patients and control subjects. With optimized assays, we measured DJ-1 and α-syn in lysate from cellular pellets obtained from the same subjects described in our previous study (Devic et al., 2011), and found that, although both proteins were easily detectable in cell pellet lysate, no significant alterations in either analyte were observed (Supplementary Fig. 1). Power analysis suggested that this sample size was sufficient to detect a moderate (0.5) effect size with 63% power at the 0.05 significance level.

Because these results suggest that the previously observed trends are largely driven by protein in the acellular component, we

Table 1
Demographic distribution and DJ-1/α-synuclein levels of subjects

	N	Age, mean (range)	DJ-1 (mean pg/μg total protein ± SEM)	α-Synuclein (mean pg/μg total protein ± SEM)
Total	198	54.9 (24–88)	184.4 ± 10.2	0.37 ± 0.02
Male	137	56.9 (24–88)	179.8 ± 11.8	0.34 ± 0.02
Female	61	50.6 (32–67)	194.8 ± 19.7	0.45 ± 0.05

Analytes reported as pg/μg total protein ± standard deviation.
Key: SEM, standard error of the mean.

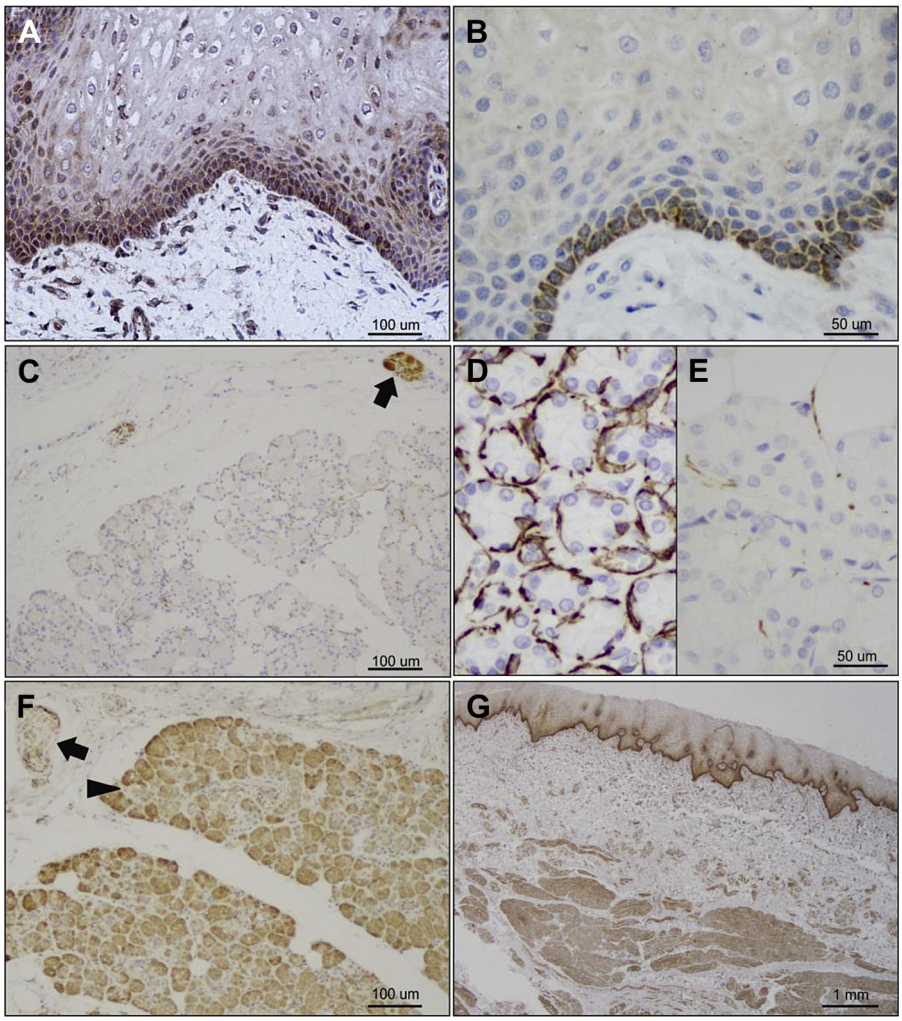

Fig. 1. (A) DJ-1 and (B) α-synuclein (α-syn) in cheek epithelium. (C) α-Synuclein in nerve in submandibular gland (SMG). Arrow indicates nerve fiber. (D) Neurofilament and (E) α-syn in SMG are localized to nerve. (F) DJ-1 is expressed throughout the SMG, including gland (arrowhead) and nerve fibers (arrow). (G) DJ-1 expression throughout cheek epithelium, nerve, muscle, and vasculature in cheek biopsy sample. All samples were collected at autopsy from subjects without neurological disease.

investigated the effects of demographic characteristics on DJ-1 and α-syn in saliva supernatants obtained from 198 healthy subjects (Table 1). Although female subjects had higher average values of both analytes, the difference was significant only for α-syn (Supplementary Fig. 2). A weak association with age was observed for DJ-1 in males, but not females or the combined group. No association with age was observed for α-syn (Supplementary Fig. 3). Tests were sufficiently powered to detect sex differences (effect size 0.5) and/or correlations with age ($R = 0.2$) with at least 80% power at a 0.05 significance level.

4. Discussion

We demonstrated the presence of DJ-1 and α-syn in cheek epithelial cells; however, no differences were found in comparing the saliva cellular components of PD patients and control subjects. This study, powered to detect moderate to large effects, cannot exclude subtle alterations, which might be revisited if larger cohorts become available. In addition, although these negative results may eliminate the possibility of cheek cells as a potential sample source for PD diagnosis, at least for total DJ-1 and α-syn, variant forms of these proteins, such as phosphorylated α-syn (e.g., pS129, the form in submandibular gland (SMG) found to be useful in detecting PD by Beach et al, 2013) or secreted protein (Devic et al., 2011) that might arise from the innervating nerve fibers in the gland, may still prove to be excellent PD biomarkers. The roles of both proteins in PD pathogenesis have been extensively investigated; however, their function in peripheral tissues, especially in non-neuronal tissues, as well as the effects of their aggregation in peripheral systems, warrant further investigation. Moreover, given the high expression of both proteins in the nerves innervating the gland, secreted forms need to be pursued preferentially in future studies. To this end, in acellular saliva from healthy subjects, we found slightly higher concentrations of both analytes in female subjects, but the difference was significant only for α-syn. We found no correlation between age and either analyte, although a small correlation was observed for DJ-1 if the group was restricted to males only. These correlations, albeit subtle, need to be considered in future investigations.

Disclosure statement

D.T.W.W. is co-founder of RNAmeTRIX Inc, a molecular diagnostic company that licenses salivary diagnostic technologies from the University of California Regents. The authors report no other conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.008.

References

Beach, T.G., Adler, C.H., Dugger, B.N., Serrano, G., Hidalgo, J., Henry-Watson, J., Shill, H.A., Sue, L.I., Sabbagh, M.N., Akiyama, H., 2013. Submandibular gland biopsy for the diagnosis of Parkinson disease. Journal of neuropathology and experimental neurology 72, 130–136.

Beach, T.G., Adler, C.H., Sue, L.I., Vedders, L., Lue, L., White III, C.L., Akiyama, H., Caviness, J.N., Shill, H.A., Sabbagh, M.N., Walker, D.G., 2010. Multi-organ distribution of phosphorylated alpha-synuclein histopathology in subjects with Lewy body disorders. Acta Neuropathol. 119, 689–702.

Cersosimo, M.G., Perandones, C., Micheli, F.E., Raina, G.B., Beron, A.M., Nasswetter, G., Radrizzani, M., Bearroch, E.E., 2010. Alpha-synuclein immunoreactivity in minor salivary gland biopsies of Parkinson's disease patients. Mov. Disord. 26, 188–190.

Del Tredici, K., Hawkes, C.H., Ghebremedhin, E., Braak, H., 2010. Lewy pathology in the submandibular gland of individuals with incidental Lewy body disease and sporadic Parkinson's disease. Acta Neuropathol. 119, 703–713.

Devic, I., Hwang, H., Edgar, J.S., Izutsu, K., Presland, R., Pan, C., Goodlett, D.R., Wang, Y., Armaly, J., Tumas, V., Zabetian, C.P., Leverenz, J.B., Shi, M., Zhang, J., 2011. Salivary alpha-synuclein and DJ-1: potential biomarkers for Parkinson's disease. Brain 134, e178.