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Towards a future molecular diagnosis of autism: Recent advances in biomarkers research from saliva samples

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ABSTRACT

Autism spectrum disorder diagnosis is currently based on clinical observations and behavioral evaluations exclusively, without any biological determination. Molecular biomarkers are usually obtained from biological fluids, such as blood or urine, generally through invasive and uncomfortable procedures. Patients with autism are characterized by sensory reactivity and behavioral difficulties which make sample collection problematic. Saliva has emerged as a feasible alternative to obtain relevant biological information and is especially indicated in the case of children with autism due to its painless and noninvasive sampling characteristics. Furthermore, saliva represents a valuable resource to study candidate biomarkers of autism. This has resulted in a number of interesting studies reported during the last 5 years that we have gathered and briefly discussed.

1. Introduction

Autism spectrum disorder (ASD) diagnosis is currently done by specialized psychologists and physicians, being exclusively based on clinical observations and behavioral evaluations, lacking molecular laboratory measurements (Ecker, 2011). This makes ASD diagnosis difficult and challenging, which affects its reliability and delays treatment. However, during the last 5 years, research in molecular medicine has revealed several candidate biomarkers that can be easily measured. The implementation of these findings into daily clinical practice could improve ASD diagnosis, making it more accurate, fast and early. Furthermore, ASD molecular biomarkers could show genetic, epigenetic and neurobiological features (Goldani et al., 2014), providing useful information for both clinicians and researchers. This will result in better understanding of its fundamentals and how it develops, as well as an improved follow-up of treatments.

Molecular biomarkers are usually determined in biological fluids, mainly from blood or urine samples. Among other signs, patients with ASD are characterized by sensory reactivity and behavioral difficulties (DuBois et al., 2017) which make sample collection problematic, being even more complex in the case of children. In contrast to traditional biofluids, saliva emerged as an interesting alternative to obtain biological samples from patients with ASD. Saliva has important advantages: it is inexpensive and easy to collect, with a painless and noninvasive procedure (Wormwood et al., 2015); has a lower anxiety-provoking effect compared to blood sample extraction and is less embarrassing than producing a urine specimen (Wormwood et al., 2015). Furthermore, saliva samples comprise a valuable source of cells and DNA (Goode et al., 2014), proteins (including cytokines, hormones, peptides, neurotransmitters and more Ratajczak and Sothern, 2015) as well as circulating microRNA (miRNA) (Gallo and Alevizos, 2013).

However, it has to be noticed that saliva sampling might have some disadvantages that have to be controlled such as contamination by interfering substances from food, beverages or oral diseases (Human, 2001). Also, some saliva biomarkers might have lower concentrations compared to blood or urine; an issue that can be solved by highly sensitive detection techniques (Wong, 2006).

In summary, all these characteristics have made saliva an appropriate biological sample to study candidate biomarkers of ASD, which has resulted in a number of interesting studies reported during the last years. The objective of this mini-review is to compile and provide a brief description of those studies and their contributions to future

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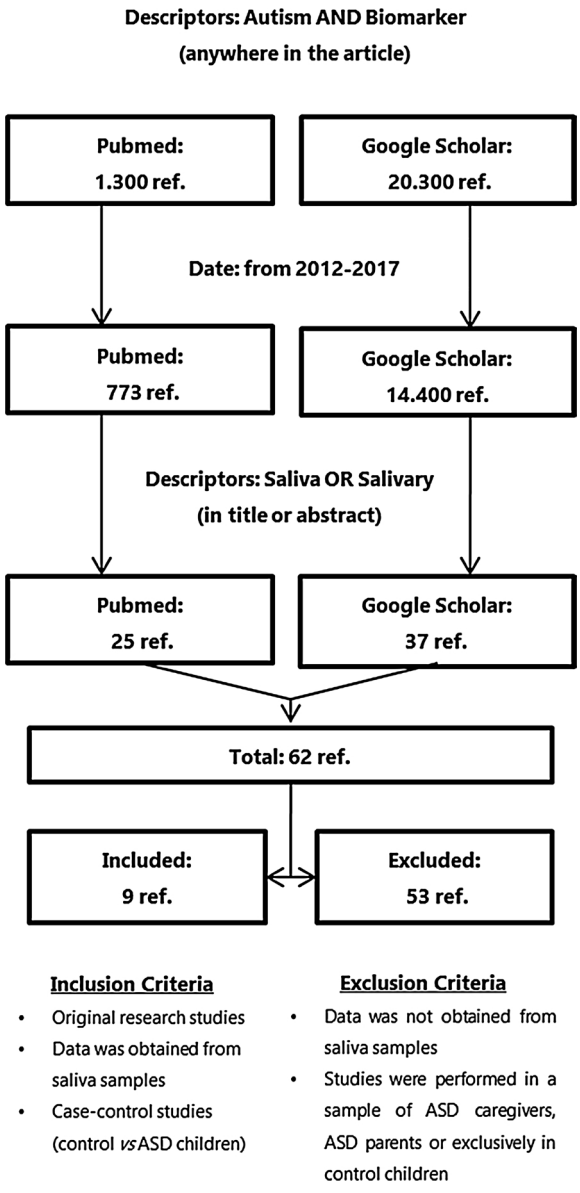

Fig. 1. Flow chart of the followed methodology.

molecular tools to improve diagnosis of ASD.

2. Material and methods

An initial search in PubMed and Google Scholar was carried out in October 2017 with descriptors "biomarker" and "autism" for papers published in peer-reviewed journals and written in English, resulting in more than 20.000 references. Papers were then filtered by publication date (from 2012 to 2017) and then, by containing the terms "saliva" or "salivary" in title or abstract, reducing the initial sample to 62 references. Finally, 9 of them met the inclusion criteria (original research studies that compared saliva samples from children with ASD *versus* control children) while 53 were excluded (data was not obtained from saliva samples or studies were performed in a sample of ASD caregivers, ASD parents or exclusively in control children, but not in ASD children). Fig. 1 shows a flowchart of the followed methodology.

Selected papers were rigorously studied and classified by sample, potential ASD biomarker and principal outcome, as shown in Table 1.

To structure results, biomarkers from selected articles were grouped into three different sections: salivary hormones, salivary proteins and salivary nucleic acids.

3. Results

3.1. Salivary hormones

3.1.1. Cortisol

High salivary diurnal cortisol levels have been related to stress and anxiety in children with ASD (Tordjman et al., 2014). For example, some studies have detected that children with ASD, compared to control children respond to threatening events by inducing an exaggerated salivary cortisol response (Corbett et al., 2006) and that cortisol elevation might be related to cognitive dysfunction (Ogawa et al., 2017), while others have not found significant changes in cortisol levels between groups (Majewska et al., 2014). Another study showed that salivary cortisol levels significantly increased and correlated to misbehavior in children with ASD during non-invasive dental procedures, compared to control children (Abdulla and Hegde, 2015). Measuring cortisol levels can be problematic due to its circadian rhythm, so values may vary depending on sample collection moment, which difficult its reliability and usefulness as a biomarker. To solve this, some authors suggest that collecting cortisol during afternoon might reduce interparticipant variability (Sharpley et al., 2016). Despite the fact that salivary cortisol levels can be determined to study the stress response in children with ASD, some published results are contradictory about its reliability and there is also no consensus regarding the best moment for saliva samples to be collected.

3.1.2. Sexual steroids

Epidemiological studies have demonstrated that ASD is 4 times more prevalent in males, suggesting a role of sex-specific biological factors such as sexual steroid hormones (Werling and Geschwind, 2013). In this context, a research group measured prenatal-amniotic and postnatal-salivary levels of testosterone and estradiol in a sample of 18–24-month-old children, concluding that amniotic testosterone, but not salivary levels, were the only variable that predicted autistic traits determined by Q-CHAT scores (Auyeung et al., 2012). Another study reported that salivary androgen levels, specifically androstenediol and dehydroepiandrosterone, as well as androgen-derived androsterone and the androgen-precursor pregnenolone were increased in ASD compared to control population, predicting early puberty (Majewska et al., 2014). Given that most of the upregulated hormones from that study are known to be neuroactive (modulating GABA, glutamate and opioid neurotransmission), the authors concluded that they could have affected brain development and its functioning, contributing to ASD development (Majewska et al., 2014). To our knowledge, these are the only currently published studies that quantify sexual hormones in saliva from confirmed or suspected children with ASD.

3.2. Salivary proteins

Biofluids proteomic analysis brings useful and relevant clinical information about both adult (Csosz et al., 2017) and pediatric diseases (Hassaneen and Maron, 2017). Research in salivary-proteomics has revealed elevated concentrations of immune-system-related proteins such as lactotransferrin, prolactin-inducible protein, neutrophil-defensin 1 or Ig kappa chain C region (Ngounou et al., 2015a). The same research team published another similar study were proteins involved in oxidative stress and lipid metabolism, such as apolipoproteins A1 and A4 and Zn alpha2 glycoprotein, were also dysregulated in ASD samples compared to control children (Ngounou et al., 2015b). These findings are consistent with the possibility of an altered immunity, oxidative stress and lipid metabolism in ASD patients (Scott et al., 2017; McGinnis, 2004; Tamiji and Crawford, 2010), that could be detectable in saliva samples by measuring the aforementioned candidate biomarkers.