INTRODUCTION

Turner syndrome is a complex genetic disorder affecting 1/2,500 live-born girls, and is caused by complete or partial absence of an X chromosome (Bondy, 2007). In Turner syndrome, at least half of the patients have a chromosome mosaic (Gravholt et al., 2006; Mortensen, Andersen, & Gravholt, 2012). Mosaicism is defined as the presence of two or more populations of different genotypes in one individual that have developed from a single fertilized egg (Strachan & Read, 2011). When and where the alterations occur in the embryonic development is of importance for which tissues and organs will be affected and to what extent. The degree of mosaicism commonly varies...
between different types of tissue and even within the same organ (Biesecker & Spinner, 2013; El-Mansoury et al., 2007; Forsberg, Gisselsson, & Dumanski, 2017; Hanson, Bryman, Janson, Jakobsen, & Hanson, 2002).

When Turner syndrome is suspected in a patient, a peripheral blood karyotype is the gold standard to confirm the diagnosis. Mosaicism is associated with a milder phenotype (El-Mansoury et al., 2007; Noordman et al., 2018), but a mosaic cell line with material from chromosome Y is associated with an increased risk of gonadoblastoma (Gravholt, Fedder, Naeraa, & Muller, 2000). As the degree of mosaicism commonly varies between different types of tissue, careful investigations of other tissues in addition to blood are essential for a correct assessment. Fluorescence in situ hybridization (FISH) of buccal cells is often used, as the FISH method is relatively inexpensive and the sampling of buccal cells is noninvasive. It is not known whether differences in the degree of mosaicism or between samples exist within the left- and right-hand sides of the buccal mucosa.

The aim was to investigate if there were any side differences in the degree of mosaicism of the buccal mucosa in women with Turner syndrome.

2 | MATERIALS AND METHODS

2.1 | Participants

The study participants were included in an ongoing genotype-phenotype protocol of women with Turner syndrome at the Turner Center at Sahlgrenska University Hospital, Gothenburg, Sweden (examined by I.B. and K.L.-W). The study was approved by the Regional Ethical Review Board in Gothenburg. All participants gave their written informed consent. The inclusion criteria were phenotypic subjects with Turner syndrome, ≥16 years of age, with partial or complete absence of an X chromosome in at least 5% of the leukocytes or buccal cells, with buccal smears taken on both the left- and right-hand sides at the same point of time. A total of 25 patients were included, 12 with mosaicism and 13 without mosaicism, based on the blood karyotype. A buccal smear from one female control without Turner syndrome was also analyzed.

2.2 | Karyotypes

The genotype was defined on the karyotype based on conventional cytogenetic analysis of G-banded metaphases after 72–96 hr of lymphocyte growth in blood. Analyses were performed of ≥25 metaphases with a chromosome quality of 400 bands according to the international guidelines for Turner syndrome (Gravholt et al., 2017). Information about karyotypes was obtained from the study protocol.

2.3 | Fluorescence in situ hybridization (FISH)

Buccal smears were taken on both the left- and right-hand sides, one slide for each side, at the same point of time for genetic analyses with FISH. Centromere probes for chromosome X (DXZ1) and Y (DYZ3) (VYSIS Inc. USA) were used. The FISH procedure that was conducted has previously been described by (Hanson et al., 2001) and ≥100 cells were evaluated. With 100 analyzed cells, the lowest level of mosaicism excluded with 99% confidence is 5% (Hook, 1977). The proportion of chromosomally abnormal cells was calculated. The same geneticists (S.T and C.H.) processed and evaluated all samples in the same laboratory and using the same methodology. The intervariability between the geneticists’ assessments of the slides was <5%. Due to the limitations of the FISH method, a difference in the degree of mosaicism ≥5% between the slides was considered as an actual difference and 5% as equivalent.

2.4 | Statistics

Continuous variables were described with means or medians and ranges, where applicable. Fisher’s exact test was used to compare differences in dichotomous variables between groups.

3 | RESULTS

A total of 12 patients with mosaicism and 13 without mosaicism, based on the blood karyotype, and one female control without Turner syndrome were evaluated. Of the 25 patients, five patients (two with mosaicism and three without mosaicism) were excluded since their slides contained <100 cells and could therefore not be evaluated.

In women with Turner syndrome, 10/20 (50%) had a ≥5% difference in mosaic degree between the right- and left-hand sides of the buccal mucosa (Figure 1). The mean difference was 9.1% and the median 4.5%, range 1%–38%. The patient who had the largest difference had a blood karyotype of 45,X/46,X,+mar(Y) with 33% 45,X on the right-hand side and 71% 45,X on the left-hand side. The control subject had no difference between the sides (0% 45,X on the left side and 0% 45,X on the right side). Of the 10 patients who were considered to have an actual difference (i.e., ≥5%) of the buccal mucosa, six had mosaicism and four did not have mosaicism in the blood based on the blood karyotype (Table 1).

4 | DISCUSSION

In this study, half of the women with Turner syndrome had a ≥5% difference in mosaic degree between the right- and left-hand sides of the buccal mucosa, regardless of whether
the patient had mosaicism or not in the blood karyotype. The difference in the mosaicism was up to 38% between the two sides of the oral cavity. This variability was nil in the woman without Turner syndrome who served as a control.

Post-zygotic variation in humans can range from an alteration of a single nucleotide to aberrations at the level of an entire chromosome and be interorgan (i.e., different genotypes in different organs) or interorgan (i.e., different genotypes in the same organ) (Biesecker & Spinner, 2013; Forsberg et al., 2017). In Turner syndrome, intraorgan mosaicism has been reported in the ovaries (Hanson et al., 2002; Landin-Wilhelmsen, Bryman, Hanson, & Hanson, 2004), but to the best of our knowledge, there are no previous reports of interorgan mosaicism in the buccal mucosa.

The finding of intraorgan mosaicism in the buccal mucosa in women with Turner syndrome is new but not unexpected, since mosaicism is a common phenomenon in different tissues and organs (Ciavarella et al., 2018; Happle, 1991; Piotrowski et al., 2008). The extent of the mosaicism is dependent on when in the embryonic development the alterations occur, with more tissues affected at an early stage. The underlying cause of the difference in the degree of mosaicism between the left- and right-hand sides of the buccal mucosa in this study is unclear. It might reflect a segmental pattern, as in cutaneous mosaicism, since both the buccal mucosa and the skin derive from the same germ layer (ectoderm) (Casale & Giwa, 2019; Chen, Jacox, Saldanha, & Sive, 2017).

It is important to detect all patients with Turner syndrome with a Y chromosome fragment as they have an approximately 10% risk of gonadoblastoma (Gravholt et al., 2017). The blood karyotype 45,X does not rule out the existence of a cryptic Y fragment. According to the clinical guidelines, it is recommended to perform FISH analyzes of at least two types

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**TABLE 1** Blood karyotype of all women with Turner syndrome and the control without Turner syndrome. The participants’ number corresponds to the number in Figure 1 showing the difference in the degree of mosaicism between the right and the left buccal mucosa, respectively

| Participant | Blood karyotype       |
|-------------|-----------------------|
| c           | 46,XX                 |
| 1           | 45,X                  |
| 2           | 45,X                  |
| 3           | 45,X/46,X,r(X)        |
| 4           | 45,X                  |
| 5           | 45,X                  |
| 6           | 45,X                  |
| 7           | 45,X                  |
| 8           | 45,X/46,XY            |
| 9           | 45,X/46,X,i(X)(q10)   |
| 10          | 45,X/46,XX            |
| 11          | 45,X                  |
| 12          | 45,X                  |
| 13          | 45,X/46,X,t(X;q)(pter:q11) |
| 14          | 45,X/46,X,der(Y)      |
| 15          | 45,X/46,X,i(Y)(p10)   |
| 16          | 45,X/46,XY            |
| 17          | 45,X                  |
| 18          | 45,X/46,XX            |
| 19          | 45,X                  |
| 20          | 45,X/46,X,+mar(Y)     |

*Abbreviations:* c, control; der, derivative chromosome; i, isocentric chromosome; mar, marker chromosome; p, short arm; pter, terminal end of the short arm; q, long arm; r, ring chromosome; t, translocation.
of tissue if virilization is present in the patient (Gravholt et al., 2017). The findings in this study of intraorganic mosaicism in the buccal mucosa indicate that FISH for chromosome Y on cells from both the left- and right-hand sides of the buccal mucosa should be considered, since a cryptic Y fragment could otherwise remain undetected.

Furthermore, the degree of mosaicism has been reported to change over time in patients with Turner syndrome (Denes, Landin-Wilhelmsen, Wettergren, Bryman, & Hanson, 2015). The present study adds new knowledge beyond that. It is of great importance to collect samples from the same type of tissue as well as the same location to get a reliable result from different time periods.

4.1 | Strength and limitations

The sample procedure and the analyses have been standardized and the same geneticists have processed and evaluated all the samples. The FISH method has limitations. However, ≥100 cells were evaluated to get a high sensitivity. A limitation of the study is the relatively small sample size. Despite the small number of study participants, a difference of ≥5% in the degree of mosaicism between the right- and the left-hand sides of the buccal mucosa could be detected in as many as 50% of the patients. Since this was detected despite the small sample size, intraorganic mosaicism in the buccal mucosa is probably highly prevalent. Besides, no fluctuation was seen at all in the right or left side, both 0%, respectively, of the buccal cells in the woman with an ordinary female karyotype without Turner syndrome who served as a control. Further studies with larger sample sizes are needed to verify these findings.

5 | CONCLUSION

This study shows that there could be intraorganic mosaicism with a difference in the degree of mosaicism of up to 38% between the left- and the right-hand sides of the buccal mucosa in women with Turner syndrome. This is important to bear in mind when taking a second sample from another tissue type as a complement to the lymphocyte sample for further evaluation of the chromosomal pattern. A cryptic Y fragment may be undetected in virilized women who are at risk of gonadoblastoma, if only one side of the buccal mucosa is analyzed with FISH. It is therefore recommended to analyze buccal smears from both sides in these cases. It is also important to collect samples from the same localization when the degree of mosaicism is studied over time.

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CONFLICT OF INTEREST

None declared.

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