# Methylation status of X inactivation-escape genes in controls and females with X chromosome rearrangements

Sayaka Kawashima[1,2], Keiko Matsubara[1], Machiko Toki[3], Rika Kosaki[4], Yukihiro Hasegawa[5], Maki Fukami[1], Masayo Kagami[1]
1 Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan
2 Department of Pediatrics, Tohoku University School of Medicine, Sendai, Japan.
3 Department of Pediatrics, Hiratsuka City Hospital, Hiratsuka, Japan
4 Division of Medical Genetics, National Center for Child Health and Development, Tokyo, Japan
5 Division of Endocrinology and Metabolism Tokyo Metropolitan Children's Medical Center, Tokyo, Japan

## Introduction and objective

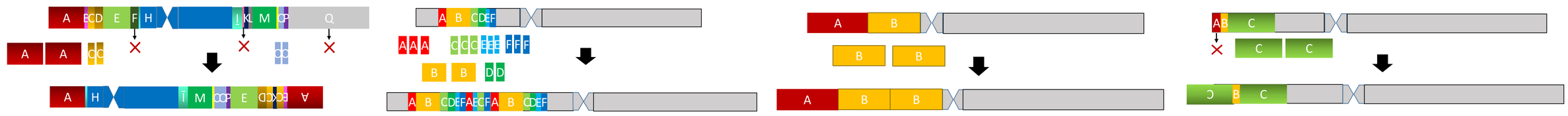
- X chromosome inactivation (XCI) is a process in which one of the two X chromosomes in females is randomly inactivated in order to correct the imbalance of gene dosage between males and females. However, about 15% of genes on the inactivated X chromosome escape from XCI [1)].
- The mechanism of inactivation and escape remains to be revealed.
- The promoter regions of escape genes are hypomethylated compared to those of the inactivated genes[2)].
- The objective of this study is to reveal the influence on the methylation status of escape genes' promoters in patients with X chromosome rearrangements.

| | XX | | | XY |
|---|---|---|---|---|
| | Inactivated X chromosome | | Activated X chromosome | X chromosome |
| | inactivated genes | Escape genes | | |
| Expression | - | + | + | + |
| Methylation status at promoter | hyper - methylation | hypo-methylation | hypo-methylation | hypo-methylation |

## Methods

**Subjects** Four patients (XX) with X chromosome rearrangements and 11 female and 12 male controls

Patient 1 / Patient 2 / Patient 3 / Patient 4



**Methods**

We performed the array-based methylation analysis with genomic DNA from leukocytes of the patients and controls using Infinium MethylationEPIC BeadChip.

**Extract escape genes**

We extracted the genes that have the hypomethylated promoter regions in both sexes.

- Within 1 kb up and downstream of transcription start sites
- Hypomethylation in both sexes (β levels < 0.15)
- Methylation differences between males and females (Δ|β|) <0.1

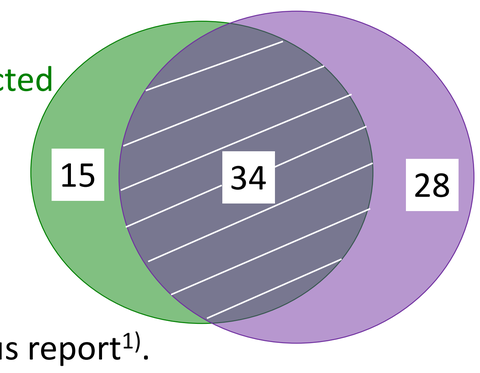
When the above items are satisfied with 2 probes or more per a gene, the gene was regarded as an escape gene.

**Methylation status in patients with X chromosome rearrangements**

We evaluated 34 genes that were predicted to be escape genes by our criteria and also reported as escape genes in the previous report[1)].

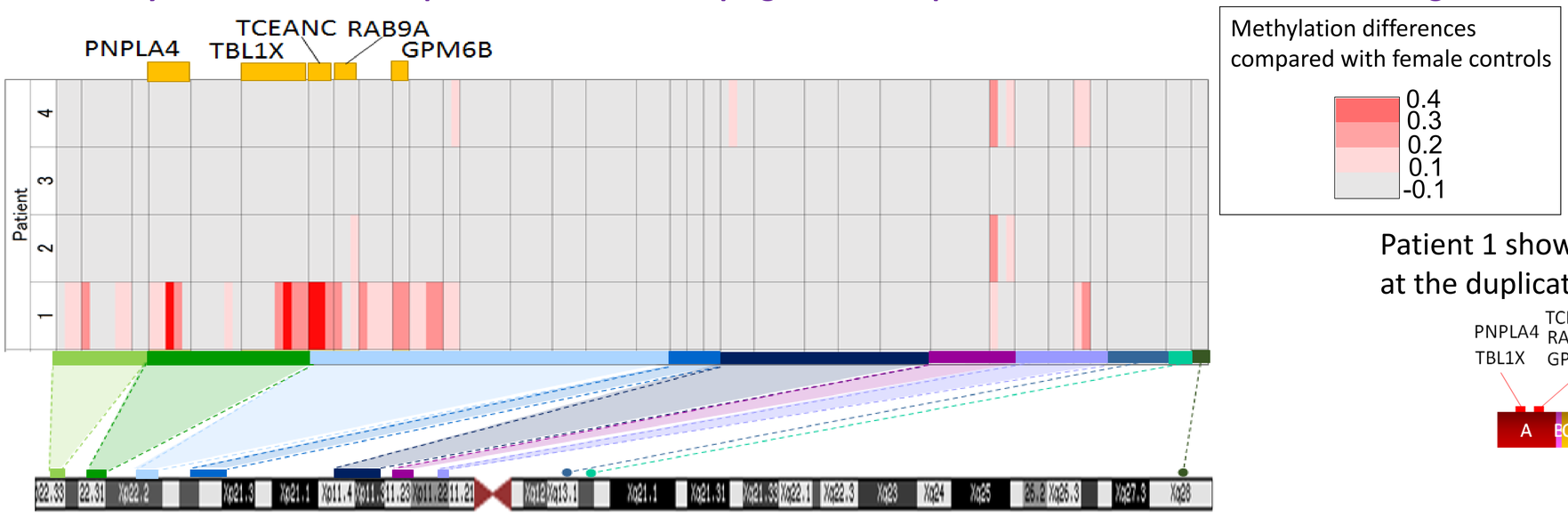
We extracted escape genes in patients satisfying the following conditions :

- β levels > 0.25
- SD > 2.0 (compared with female controls)
- The above items are satisfied with 2 probes or more per a gene



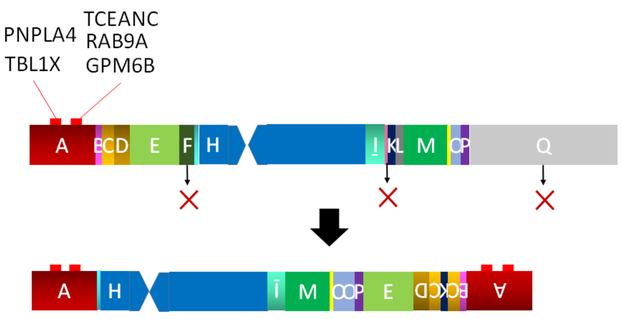

## Results

**The methylation status of the promoters of the escape genes in the patients with X chromosome rearrangements**



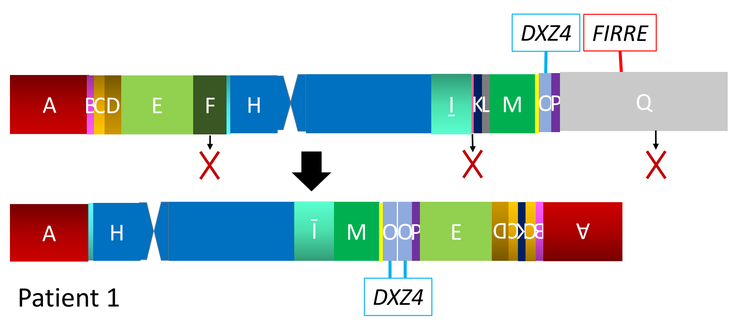

: The escape genes that showed methylation abnormalities in patient 1

Patient 1 showed hypermethylation in 5 escape genes at the duplicated region on short arm.




## Discussion

- One patient showed the elevated methylation levels at the promoter regions of some escape genes.
- This finding suggests that structural abnormalities on X chromosome can affect the methylation levels of the promoter regions in some escape genes.
- This patient has a deletion or a duplication of the important loci to form 3D structure of inactivated X chromosome (*FIRRE* and *DXZ4* respectively). Recently, it was reported that deletion of *Firre* and *Dxz4* in mouse ES cells led to the change in gene expression of some escape genes.[3)] The methylation change in the patient may be caused by the deletion of FIRRE and/or the duplication of *DXZ4*.




## Conclusion

Specific X chromosome rearrangements is likely to affect the methylation status of promoter regions of some escape genes.

1) Carel. L, Willard. H. F *Nature* 2005;434 2) Cotton A et al. *Hum Mol Genet* 2015; 24 3) Froberg JE et al. *Nat Commun*. 2018;9

58ESPE P1-140
Sex differentiation, gonads and gynaecology or sex endocrinology
Sayaka Kawashima
Poster presented at: ESPE 2018 VIENNA
PosterSessionOnline.com