They described that all were of paternal origin and spermatogenesis is more prone to generate multiple chaotic chromosome imbalances and reciprocal translocations than oogenesis. Replication and/or repair systems in spermatogenesis might differ from those in oocytogenesis and be associated with paternal bias of polyalanine expansion mutation.

There are a small number of asymptomatic or rarely symptomatic individuals with somatic mosaicism. Regarding the origin of polyalanine expansion in the somatic mosaicism, we studied the genome of an individual (a mother of a patient) with somatic mosaicism of 7-alanine expanded (27-alanine) and wild (20-alanine) alleles, but were unable to detect a 7-alanine contracted (13-alanine) allele, a counter allele of the expanded allele, which should have been produced at the recombination event (data not shown). Other groups also did not find a counter polyalanine contracted allele in the genome of individuals with somatic mosaicism.[9,10] Polyalanine expansion in somatic mosaicism is unlikely to have been derived from recombination. Trochet *et al.*[9] reported three rare complex expansion mutations of *PHOX2B* and suggested that mechanisms other than an unequal crossing-over model would be involved. One of the three mutations is explained by recombination involving the codon encoding glycine 240 located 5′-upstream of the sequences encoding the polyalanine tract. However, two other complex expansion mutations are not explained by the recombination mechanism. Polyalanine expansion mutations in somatic mosaicism and few complex expansion mutations cannot be derived from unequal sister chromatid exchange, but are explainable by a replication mechanism such as a model of a repetitive hairpin formation on the nascent strand or a model of repeat instability generated during replication fork stalling and restart within the repetitive run.[20]

The results of our study suggest that unequal sister chromatid exchange during spermatogenesis is a major cause of *de novo* polyalanine expansion mutations, except for rare complex mutations and expansion mutations in somatic mosaicism.

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1 Amiel, J., Laudier, B., Attie-Bitach, T., Trang, H., de Pontual, L., Gener, B. *et al.* Polyalanine expansion and frameshift mutations of the paired-like homeobox gene *PHOX2B* in congenital central hypoventilation syndrome. *Nat. Genet.* **33,** 459–461 (2003).
2 Sasaki, A., Kanai, M., Kijima, K., Akaba, K., Hashimoto, M., Hasegawa, H. *et al.* Molecular analysis of congenital central hypoventilation syndrome. *Hum. Genet.* **114,** 22–26 (2003).
3 Weese-Mayer, D. E., Berry-Kravis, E. M., Zhou, L., Maher, B. S., Silvestri, J. M., Curran, M. E. *et al.* Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in PHOX2b. *Am. J. Med. Genet. A* **123A,** 267–278 (2003).
4 Matera, I., Bachetti, T., Puppo, F., Di Duca, M., Morandi, F., Casiraghi, G. M. *et al.* PHOX2B mutations and polyalanine expansions correlate with the severity of the respiratory phenotype and associated symptoms in both congenital and late onset central hypoventilation syndrome. *J. Med. Genet.* **41,** 373–380 (2004).
5 Trochet, D., Hong, S. J., Lim, J. K., Brunet, J. F., Munnich, A., Kim, K. S. *et al.* Molecular consequences of PHOX2B missense, frameshift and alanine expansion mutations leading to autonomic dysfunction. *Hum. Mol. Genet.* **14,** 3697–3708 (2005).
6 Berry-Kravis, E. M., Zhou, L., Rand, C. M. & Weese-Mayer, D. E. Congenital central hypoventilation syndrome: PHOX2B mutations and phenotype. *Am. J. Respir. Crit. Care Med.* **174,** 1139–1144 (2006).
7 Warren, S. T. Polyalanine expansion in synpolydactyly might result from unequal crossing-over of HOXD13. *Science* **275,** 408–409 (1997).
8 Arai, H., Otagiri, T., Sasaki, A., Hashimoto, T., Umetsu, K., Tokunaga, K. *et al. De novo* polyalanine expansion of PHOX2B in congenital central hypoventilation syndrome: unequal sister chromatid exchange during paternal gametogenesis. *J. Hum. Genet.* **52,** 921–925 (2007).
9 Trochet, D., de Pontual, L., Keren, B., Munnich, A., Vekemans, M., Lyonnet, S. *et al.* Polyalanine expansions might not result from unequal crossing-over. *Hum. Mutat.* **28,** 1043–1044 (2007).
10 Parodi, S., Bachetti, T., Lantieri, F., Di Duca, M., Santamaria, G., Ottonello, G. *et al.* Parental origin and somatic mosaicism of PHOX2B mutations in congenital central hypoventilation syndrome. *Hum. Mutat.* **29,** 206 (2008).
11 Horiuchi, H., Sasaki, A., Osawa, M., Kijima, K., Ino, Y., Matoba, R. *et al.* Sensitive detection of polyalanine expansions in PHOX2B by polymerase chain reaction using bisulfite-converted DNA. *J. Mol. Diagn.* **7,** 638–640 (2005).
12 Wells, R. D., Dere, R., Hebert, M. L., Napierala, M. & Son, L. S. Advances in mechanisms of genetic instability related to hereditary neurological diseases. *Nucleic Acids Res.* **33,** 3785–3798 (2005).
13 Yoon, S. R., Dubeau, L., de Young, M., Wexler, N. S. & Arnheim, N. Huntington disease expansion mutations in humans can occur before meiosis is completed. *Proc. Natl Acad. Sci. USA* **100,** 8834–8838 (2003).
14 Hu, X. Y., Ray, P. N. & Worton, R. G. Mechanisms of tandem duplication in the Duchenne muscular dystrophy gene include both homologous and nonhomologous intrachromosomal recombination. *EMBO J.* **10,** 2471–2477 (1991).
15 Crow, J. F. The origins, patterns and implications of human spontaneous mutation. *Nat. Rev. Genet.* **1,** 40–47 (2000).
16 Ira, G., Malkova, A., Liberi, G., Foiani, M. & Haber, J. E. Srs2 and Sgs1-Top3 suppress crossovers during double-strand break repair in yeast. *Cell* **115,** 401–411 (2003).
17 Prakash, R ., Satory, D ., Dray, E ., Papusha, A ., Scheller, J., Kramer, W. *et al.* Yeast Mph1 helicase dissociates Rad51-made D-loops: implications for crossover control in mitotic recombination. *Genes Dev.* **23,** 67–79 (2009).
18 Onoda, F., Seki, M., Wang, W. & Enomoto, T. The hyper unequal sister chromatid recombination in an sgs1 mutant of budding yeast requires MSH2. *DNA Repair (Amst)* **3,** 1355–1362 (2004).
19 De Gregori, M., Ciccone, R., Magini, P., Pramparo, T., Gimelli, S., Messa, J. *et al.* Cryptic deletions are a common finding in 'balanced' reciprocal and complex chromosome rearrangements: a study of 59 patients. *J. Med. Genet.* **44,** 750–762 (2007).
20 Mirkin, S. M. Expandable DNA repeats and human disease. *Nature* **447,** 932–940 (2007).