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Selective chromatid segregation mechanism proposed for the human split hand/foot malformation development by chromosome 2 translocations: A perspective

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ABSTRACT

Three unrelated chromosome 2q14.1–14.2 region translocations caused the split hand/foot limb malformation development in humans by an unknown mechanism. Their etiology was described by the autosomal dominant inheritance with incomplete penetrance genetic model although authors stated, "the understanding of the genotype-to-phenotype relationship has been most challenging". The conundrums are that no mutation was found in known genes located at or near the translocation breakpoints, some limbs were malformed while others were not in the same patient and surprisingly breakpoints lie at relatively large distance of more than 2.5 million bases to have caused disorder-causing gene mutations in a single gene. To help understand translocations etiology for limb development, we invoke the selective DNA strand/chromatid-specific epigenetic imprinting and segregation mechanism employed by the two highly diverged fission yeasts to produce daughter cells of different cell types by mitosis. By this mechanism, an anterior- and posterior-limb-tissues-generating pair of daughter cells is produced by a single deterministic cell dividing in the anlagen of the limb bud. Accordingly, malformation develops simply because translocations hinder the proper distribution of chromatid-specific epialleles of a limb developmental gene during the deterministic cell's mitosis. It is tempting to speculate that such a mechanism might involve the *HOXD*-cluster genes situated centromere-distal to the translocation breakpoints many million bases away at the 2q31.1 region. Further genetic tests of the hypothesis are proposed for the human and mouse limb development. In sum, genetic analysis of translocations suggests that the sequence asymmetry of strands in the double-helical DNA structure of a developmental gene forms the physical basis of daughter cells' developmental asymmetry, thus opposing the morphogen-gradient research paradigm of limb development.

1. Background

"The two big problems – the nature of development and the nature of mind – are being subdued. I don't know whether there will be beautiful, general theories to come out of this – something really nice like Watson and Crick's double helix – or whether there will be an accumulation of more and more details. I'll confess to a secret hope for the former (Crow, 2000)."

Limb development has been one of the most active areas of developmental biology research spanning more than 100 years (reviewed in Zeller et al., (2009)). The morphogen gradient model (Wolpert, 1969) has been the primary paradigm used for guiding research for limb and other organs development during embryogenesis. Limb development involves cell growth regulation, cell-type differentiation and cell death by a series of signaling cascades, such as the sonic hedgehog signaling, the bone morphogenetic and the fibroblasts growth factors pathways. How these molecular mechanisms originate only in specific cells and act temporally in time and space in developing tissues of the limb bud are not understood, as a result, this system continues to be a very active area of research aimed at discovering embryonic developmental mechanisms. Little is known about how development of different cell types and tissues are coordinated to form an organ, such as the limb. As one of the approaches, spontaneously arising human mutations affecting limb development have been exploited as a target of research for deciphering developmental mechanisms. For

Abbreviations: SHFM, congenital split hand/foot malformation; Chr., chromosome; SSIS, somatic strand-specific imprinting and selective sister chromatid segregation mechanism; Mb, magabases; ANT1, anterior/posterior limb tissues fates-determining gene; ON, transcriptionally active *ANT1* gene epiallele; OFF, epigenetically silenced *ANT1* epiallele

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example, the split hand/foot malformation (SHFM; OMIM 225300), also referred to as ectrodactyly, is a prominent congenital defect in limb digits formation. It consists of a spectrum of limb malformations of distal portions of the hand/foot due to a deep median cleft, missing digits and bones, such as the split hand/foot long-bone deficiency (SHFLD) malformation. For our discussion here these malformations will be collectively referred to as the SHFM disorders. They occur in about 1 in 18,000 births and most cases are sporadic in nature of unknown etiology (Czeizel et al., 1993). Also, much variability in the malformation phenotype exists, and inexplicably, some limbs are malformed while others are not in the same genetically predisposed individual.

A long history of research on limb developmental anomalies has revealed at least six loci, mutations of which are thought to cause the developmental disorder in humans (reviewed in (Babbs et al., 2007; Bernardini et al., 2008; David et al., 2009; Elliott and Evans, 2006; Scherer et al., 1994; Sowinska-Seidler et al., 2014; van Silfhout et al., 2009)). These loci represent several chromosomes (Chr.): *SHFM1* maps on Chr. 7q21 (MIM 183600); *SHFM2* on Chr. Xq26 (MIM 313350); *SHFM3* on Chr. 10q24 (MIM 600095); *SHFM4* on Chr. 3q27 (MIM 605289), and *SHFM5* on Chr. 2q31.

This perspective is limited to addressing the puzzling genetic behavior of another locus represented by three Chr. 2q14.1–q14.2 regions balanced translocations involving three other chromosomes. A patient with SHFLD carried a *de novo* chromosomal translocation t(2;18)(q14.1;p11.2), and all limbs were affected (Babbs et al., 2007). A proband carried a *de novo* reciprocal t(2;11)(q14.2;q14.2) translocation where only the feet were malformed (David et al., 2009). Another *de novo* t(2;4)(q14.1;q35) translocation was associated with the SHFM disorder where only the feet were affected (Corona-Rivera et al., 2000; David et al., 2009). Because the pathogenesis of SHFM/SHFLD syndrome caused specifically by Chr. 2 translocations is not understood (Babbs et al., 2007; Corona-Rivera et al., 2000; David et al., 2009; Duijf et al., 2003), this topic comprises the subject of this perspective paper. Here we advocate the selective chromatid segregation mechanism, previously researched and discovered to operate in two fission yeasts, for controlling spatial and temporal regulation of gene expression required for limb development. In short, a new hypothesis is proposed for how a genetic mechanism could impact a puzzling birth defect syndrome and the role of chromatid segregation in developmental biology, with SHFM as one of the major examples is described.

2. The genetic mechanisms of SHFM caused by Chr. 2 translocations remain elusive

Because all three translocations involve Chr. 2 and the *Dominant hemimelia* mouse mutation causing SHFM map to a Chr. 1 region syntenic with humans 2q14.2 region, this region was proposed to represent the *SHFLD* locus in humans (Babbs et al., 2007). However, David and colleagues (David et al., 2009) were surprised to find that the breakpoints of three translocations mapped as far as 2.5 megabases (Mb) apart at the 118.4 Mb (q14.1) and 120.9 Mb (q14.2) regions of Chr. 2. The second surprise was that none of the breakpoints of all three translocations disrupted a known gene, and third, no clear pathogenic mutations were identified in any of the genes located near the breakpoints (Babbs et al., 2007; David et al., 2009). The fourth surprise was that some limbs in translocation carriers were malformed while others were unaffected even within the same individual. Thus, inexplicably, the mutation formally acts dominant to the wild type gene in some limbs but recessive in others. In the studies cited above, 8 limbs of the translocation-harboring persons were malformed while 4 were unaffected. These studies hypothesized that the separation of long-range, *cis*-acting regulatory elements by translocations causes the disruption of precise quantitative expression of one or more genes at the breakpoint region and/or that the breakpoints affected the function of different genes at or near each breakpoint to cause malformation. However, the mechanisms of altered gene dosage by Chr. 2 translocations remain to be defined. Moreover, except for the cases of the p63 transcription factor′s mutations of the *SHFM4* locus, factors causing SHFM in the majority of patients concerning other loci remain poorly understood via the prevailing morphogen gradients research paradigm (Duijf et al., 2003; Kouwenhoven et al., 2010; Sowinska-Seidler et al., 2014).

The genetic model of autosomal dominant inheritance with incomplete penetrance has best described the variable phenotypes of translocations (Babbs et al., 2007; David et al., 2009). We focus our analysis here to explain the molecular basis of the unexplained features of this model. These features include the presumed mutation's dominance to the wild-type gene in some limbs but not in others (i.e., incomplete penetrance), the absence of findings of gene mutations located at or near breakpoints and the separation of breakpoints by a relatively large distance of 2.5 Mb to have affected a single locus to cause the disorder. Since there is a significant conservation of biological mechanisms operating in the research model yeast organisms with those of metazoans, such as of mitosis, meiosis, epigenetic processes and the like, for limb development we therefore consider the chromosome-based epigenetic mechanism that we have discovered to produce sister cells of two different cell types in the haploid *Schizosaccharomyces pombe* and *Schizosaccharomyces japonicas* fission yeasts (reviewed in Klar et al. (2014)). This mechanism was subsequently advanced for explaining the visceral organs left–right axis development in mice (Klar, 1994) and for brain hemispheric laterality development in humans (Klar, 2002). This mechanism is advanced here to develop a molecular mechanism for Chr. 2 translocations etiology of the SHFM disorder. Deciphering the mechanism of limb malformations is hoped to further define the biology of limb development in humans and other animals, and additionally, it should help guide future research on development of diploid and multicellular organisms at large.

3. The somatic selective strand/chromatid-specific Imprinting and selective chromatid segregation (SSIS) mechanism advanced for producing anterior- and posterior-fated limb progenitor sister cells at a deterministic asymmetric cell division in the limb bud

The discoveries of gene-silencing phenomenon in budding yeast *Saccharomyces cerevisiae* that identified the founding member *MAR1/SIR2/Sirtuin* gene for epigenetic control (Klar, 2010; Klar et al., 1979), epigenetic differentiation of sister chromatids at the mating-type locus in two evolutionarily diverse fission yeasts ((Klar, 1987, 1990; Yu et al., 2013); reviewed in Klar et al. (2014)), the cell-type-regulated selective versus random Chr. 7 chromatids segregation phenomenon of mouse cells (Armakolas and Klar, 2006), and the discovery of autosomal chromatids selective segregation phenomenon in *Drosophila* (Sauer and Klar, 2013 ; Yadlapalli and Yamashita, 2013) have formed the basis for proposing the SSIS mechanism (Fig. 1). Yeasts execute cell-autonomous developmental decisions in individual cells, but it remains unknown whether limb bud cells acquire their developmental fates as individual cells or as groups of cells (Zeller et al., 2009). Motivated to understand the molecular basis of Chr. 2 translocations etiology, we expand here on the idea that the initial cell-fate determination occurs by developmentally regulated asymmetric cell division of a single deterministic cell dividing in the anlagen of the limb bud through the SSIS mechanism (Fig. 1). Accordingly, asymmetric