Non-coding deletions identify Maenli lncRNA as a limb-specific En1 regulator

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（1）簡述論文的重大發現

長鏈非編碼 RNA (lncRNA) 可能是 gene-regulatory networks 中的重要組成部分，可能會造成人類疾病或使造成肢體型態改變。這篇論文，發現了人類二號染色體的 lncRNA 的 genetic ablation 會導致嚴重的先天性肢體畸形。作者在具有 mesomelic shortening、syndactyly and ventral nails (dorsal dimelia)的肢體畸形患者中發現二號染色體上的 engrailed-1 基因 (EN1) 上游 300 kb 的 27 – 63 kb 有 homozygous deletions。

在小鼠中重新設計人類 homozygous deletions 會導致 En1 在肢體中的表達以及重現人類疾病表型的 double dorsal-limb 表型。在發育中的小鼠肢體中進行的全基因組轉錄組分析顯示，缺失區域內有一個四個 exon 長的 long non-coding transcript，作者將其命名為 Maenli。Maenli locus 的功能分析表明，cis 中的 transcription 會影響 En1 在肢體中的活化，Maenli 的 deletion 也會導致 En1 相關的 dorsal ventral limb 表型。

（2）對論文內容的疑問

在 Fig.2 中的 c 圖，為何在 E9.5 中 Maenli 在 En1 中還有表達，在 E10.5 中 Maenli 的表達卻沒有了？

（3）論文的缺點與評論

這篇論文的嚴謹性很高，使用了各項實驗及數據分析來證實 Maenli 的各種調控對 En1 的影響，或許在肢體畸形患者中還有一些 gonome 會影響表型，是作者尚未發現的。

Article

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Long non-coding RNAs (lncRNAs) can be important components in gene-regulatory networks[1], but the exact nature and extent of their involvement in human Mendelian disease is largely unknown. Here we show that genetic ablation of a lncRNA locus on human chromosome 2 causes a severe congenital limb malformation. We identified homozygous 27–63-kilobase deletions located 300 kilobases upstream of the engrailed-1 gene (*EN1*) in patients with a complex limb malformation featuring mesomelic shortening, syndactyly and ventral nails (dorsal dimelia). Re-engineering of the human deletions in mice resulted in a complete loss of *En1* expression in the limb and a double dorsal-limb phenotype that recapitulates the human disease phenotype. Genome-wide transcriptome analysis in the developing mouse limb revealed a four-exon-long non-coding transcript within the deleted region, which we named Maenli. Functional dissection of the *Maenli* locus showed that its transcriptional activity is required for limb-specific *En1* activation in *cis*, thereby fine-tuning the gene-regulatory networks controlling dorso-ventral polarity in the developing limb bud. Its loss results in the *En1*-related dorsal ventral limb phenotype, a subset of the full *En1*-associated phenotype. Our findings demonstrate that mutations involving lncRNA loci can result in human Mendelian disease.

There has been enormous progress in exploring disease variants in the human genome. Yet, the interpretation of variants in the non-coding genome remains a challenge owing to the myriad mechanisms by which they can potentially cause disease. Besides disrupting *cis*-regulatory elements, non-coding variants may interfere with the function of non-coding transcripts. Indeed, a substantial fraction of the human genome is transcribed into RNA, although most transcripts lack protein-coding potential and are referred to as non-coding transcripts[2]. Characterization of a small number of these RNA molecules has revealed that they may have roles as regulators of gene expression through diverse modes of action[3]. However, the identification of functional non-coding transcript loci remains challenging. Thus, annotating non-coding transcript loci and unravelling their function will substantially improve our knowledge about gene regulation and the identification and interpretation of non-coding genetic variants with respect to disease pathogenesis.

Non-coding deletions cause limb malformations

We identified 27–63-kb non-coding deletions of chromosome 2 in three unrelated individuals (patients 1–3) with a type of limb malformation that, to our knowledge, remains undescribed. Affected individuals had a severe shortening and deformation of the legs and feet, 3/4 syndactyly of the hands, as well as the presence of nails on the palmar side of fingers IV and V (Fig. 1a, Extended Data Fig. 1a, b, Supplementary Note 1). Radiographs showed normal femora but severely shortened tibiae, triangular fibulae and malformed or absent bones in the feet (Fig. 1a, Extended Data Fig. 1a, Supplementary Note 1). Exome sequencing did

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