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(別紙様式 2)
(Separate Form 2)

論文の要旨

Summary (Abstract) of doctoral thesis contents

Background

The S-100 ‘fused’ type genes (SFTP) are members of a gene family part of the Epidermal Differentiation Complex (EDC). EDC is a cluster of genes important for skin structure and were previously described as rapidly divergent. All SFTPs have a similar structure with a tandem repeated region on their third exon.

The evolutionary dynamics of repeated sequences is quite complex, with some duplicates never having differentiated from each other. Two models can explain the evolutionary process for repeated genes—concerted and birth-and-death, of which the latter is driven by purifying selection and has a high level of diversity across repeats. The result of random duplications and losses in repeated regions might modulate molecular pathways and therefore affect phenotypic characteristics in a population. The effect of repeats variation is of interest for the SFTP family as all members share a repetitive exon structure and are located on a rapidly divergent region.

Filaggrin, a member of the SFTP, contains repeat variations across and within species. In human, the filaggrin repeat number variation affects its function with fewer repeats resulting in a higher risk for atopic dermatitis (AD). Globally, Ecuador has the second highest prevalence of AD in children but no studies associated to filaggrin.

I investigated whether the variation in the number of tandem repeats could be found in all SFTPs. Next, I examined which model, concerted or birth-and-death fits best for each member of the SFTP. Finally, I searched for filaggrin variations associated to AD in pediatric Ecuadorian cases.

Materials and methods

The members of the SFTP family are cornulin (*CRNN*), filaggrin-2 (*FLG-2*), filaggrin (*FLG*), hornerin (*HRNR*), repetin (*RPTN*) and trichohyalin (*TCHH*). I obtained DNA sequences from the NCBI database for *FLG, FLG-2, RPTN* and *CRNN* of human, chimpanzee, gorilla, orangutan, and macaque, and for *HRNR* of baboon and marmoset. *TCHH* was not used because it consists of domains rather than repeats. In *FLG*, I obtained new sequences by combining short and long length-sequencing methods.

For each member of the SFTPs, I performed multiple alignment, and phylogenetic analyses across species. Next, I estimated the nucleotide variation between repeats within a species. Finally I searched for codons under selection by maximum likelihood analyses.

Ecuadorian pediatric samples were obtained from CEPI-center (Quito-Ecuador). I extracted DNA from dry blood card by a modified extraction protocol, performed PCR for the repeated region and sequenced using MiSeq system. I evaluated possible associations by using multiple logistic regression.

Results and discussion

I found that SFTP had variation in the number of repeats across species. Overall, the nucleotide variation for each gene ranged similarly and was comparable with that of birth-and-death model. In addition, most of variations are synonymous which is the driving force for the birth-and-death model. Under the birth-and-death model, duplicates, with enough divergence time, can lead to lineage-specific expansions which I observed in the phylogeny of *FLG, FLG-2, HRNR* and *CRNN*.

The nucleotide diversity and phylogeny analyses supported a previously described SFTP division. This division grouped SFTP intro three groups, filaggrin-type (*FLG, FLG-2* and *HRNR*), trichohyalin-type (TCHH and RPTN) and cornulin. Filaggrin-type had high nucleotide variation and duplicates within species, which allow variability in the CE. The trichohyalin-type had low nucleotide changes, its repetitive domains are conserved and single nucleotide variations have a phenotypic effect. Cornulin is the most conserved with the lowest nucleotide variability due to its role in cell cycle.

Additionally, I found individual findings for each SFTPs. For *FLG*, I observed length variation of the repeated region in chimpanzee and crab-eating macaque. For *HRNR*, I clarified the formation, starting with a 117-bp unit that duplicated three times and then duplicated on a sequential pattern in chimpanzee, gorilla and orangutan and on a different pattern in human. *FLG-2* had two different repeated regions that evolved separately but still under the birth-and-death model. For *CRNN*, previously was suggested to evolve under positive selection however my analysis demonstrated misalignment in that analysis and clarified purifying selection.

I analyzed the filaggrin variations in Ecuadorian pediatric population. Most cases and controls have 12-filaggrin repeats and were not associated with the risk for AD. LOF mutations are also associated with AD. The frequency of the five most common variations in Northern Europeans was low in Ecuadorians. However, I associated 2 new non-synonymous damaging variations (E2250Q and E2652D) with cases.

Conclusions

I concluded that SFTPs evolved under the birth-and-death model by showing high nucleotide diversity within a species and duplication and loss events within and across species. Also, I concluded that filaggrin repeat variation was not associated to the risk of AD in Ecuadorian children; however, I detected two new non-synonymous damaging variants associated to cases.

(別紙様式 3)
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博士論文審査結果の要旨

Summary of the results of the doctoral thesis screening

ヒトゲノムの第１番染色体には、Epidermal Differentiation Complex (EDC)と呼ばれる、皮膚の構造に重要である遺伝子群が存在する。EDC 遺伝子群には、S-100 融合タイプ遺伝子 (SFTP)とよばれる、第３エクソンに直列アミノ酸リピートを持つという類似構造を持った遺伝子グループが存在する。リピート構造をもつ SFTP には、以下の遺伝子が含まれる：cornulin、filaggrin、filaggrin-2、hornerin、repetin。出願者は、これら SFTP 遺伝子グループのうち、filaggrin についてはチンパンジー、ゴリラ、オランウータン、アカゲザル、カニクイザルにおける遺伝子配列を新規に決定し、ゲノムデータベースに掲載されているこれらの種の filaggrin 遺伝子配列が間違っていることをあきらかにしたうえで、一連の分子進化学的解析をおこなった。他の４遺伝子については、ゲノムデータベースから塩基配列を取得し、filaggrin 遺伝子と同様の分子進化学的解析をおこなった。これらの結果、リピート構造をもつ EDC 遺伝子群の進化は、リボソームＲＮＡのリピート配列でみられるような協調進化はしておらず、birth-and-death 過程にしたがっていることをあきらかにした。いくつかのアミノ酸置換は正の自然淘汰によっている可能性も指摘した。cornulin の進化については、先行研究で正の自然淘汰が生じているという結果が、配列のアラインメントミスであることを発見し、純化淘汰が生じているとした。また、出願者の出身国であるエクアドルで 329 名（atopic dermatitis の患者と対照群）のＤＮＡサンプルを収集し、filaggrin 遺伝子の変異について塩基配列決定により解析した結果、患者と対照群で、filaggrin 遺伝子に明確な差は見いだせなかったが、患者群で２個のアミノ酸変異を見いだした。

この研究は、ヒトの皮膚に重要な関与をする EDC 遺伝子群の進化と病気について、あたらしい知見を加えるものである。審査員全員で審査した結果、本大学院における学位授与の水準を満たす論文であると判断した。