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DR. KHOO TENG LYE
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The Korean Association of Plastic Surgeons(KAPS) &
International Aesthetic Expo 2012

Contributions of TGFβ1 and SMAD4 Genes to the Etiology of Keloid Scars in the Malay Population

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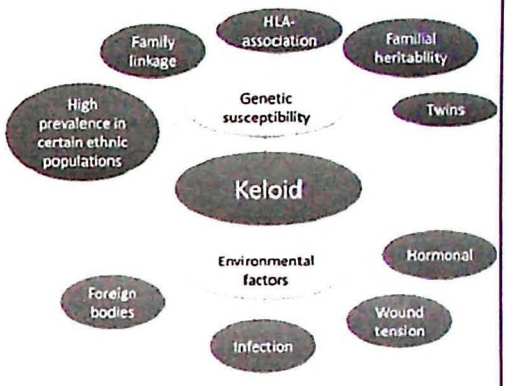
Background

- Keloid scars are complex dermal condition
  - excessive deposition of extracellular matrix
  - especially collagen

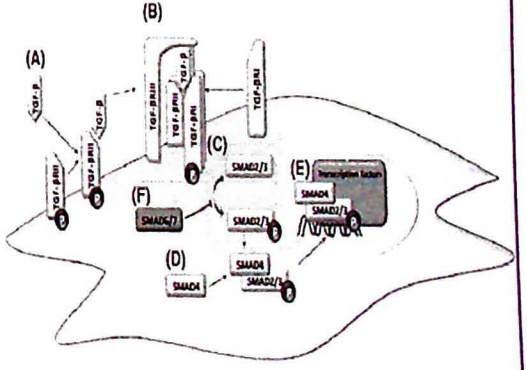
Background

- Keloid scars
  - genetic and
  - environmental contributing factors.
- High prevalence among certain ethnicities and familial aggregation has provided evidence for genetic risk factors.



Background

- *TGFβ*1
  - is highly expressed in keloid fibroblast cells (Peltonen *et al.*, 1991, Lee *et al.*, 1999)
  - regulates the expression of several downstream genes.

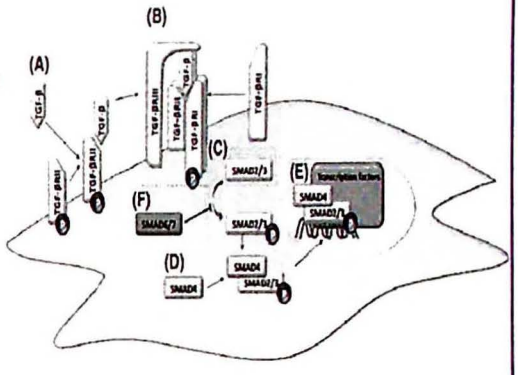


TGFB pathway in which *SMAD* genes act downstream to transfer *TGFB* signals into the nucleus
Adapted from (Shih and Bayat, 2010).

Background

SMAD4

- located on chromosome 18q21.1
- has also been associated with keloid disease in a Chinese population (Yan *et al.*, 2007b).



TGFB pathway in which *SMAD* genes act downstream to transfer *TGFB* signals into the nucleus
Adapted from (Shih and Bayat, 2010).

Background

- To date
  - only few documented reports showing relationship between TGFβ1 gene and keloid in Caucasian population (Bayat *et al.*, 2003b)
  - but none on SMAD4 gene.

Purpose

- To study *t*he association
  - between variants of TGFβ1 and SMAD4 genes
  - in the keloid formation
  - of Malay population

Subjects and Methodology

- The DNAs were extracted from the blood samples of
  - 100 Malay patients with keloids
  - 100 healthy Malay individuals without keloids as controls.

Subjects and Methodology

Case group

- **Inclusion criteria**

1. Malay population with keloids
2. Age: 10-55

Subjects and Methodology

Case group

- **Exclusion criteria**

1. Skin disorders with keloid
2. Syndromic disorders with keloid
3. Subjects with other disorders

   e.g. mental retardation, chromosomal disorders, etc

Subjects and Methodology

Control group (Healthy volunteers)

- **Inclusion criteria**

1. Normal healthy people
2. Matched ethnicity, age and sex with the study group

Subjects and Methodology

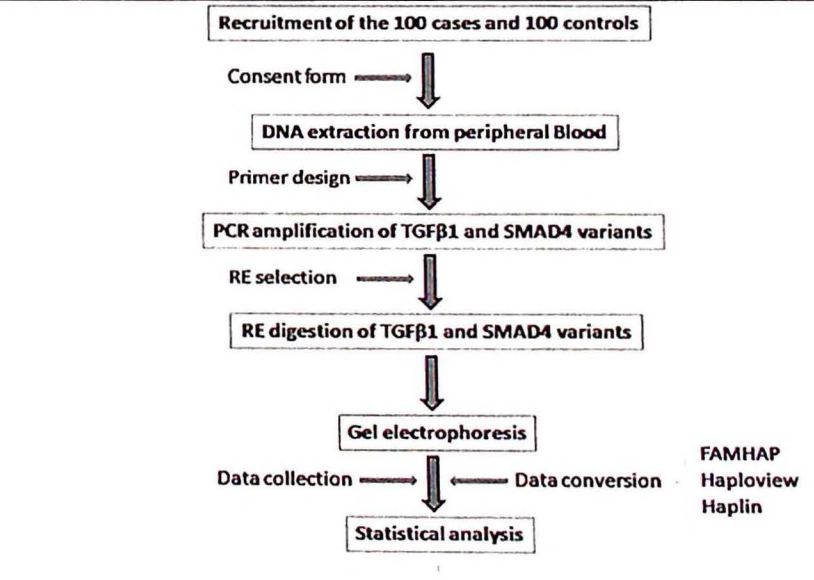
Control group (Healthy volunteers)

- **Exclusion criteria**

1. history of keloids, Hypertrophic scar or skin disorders
2. mixed ethnic parentage or unknown parentage

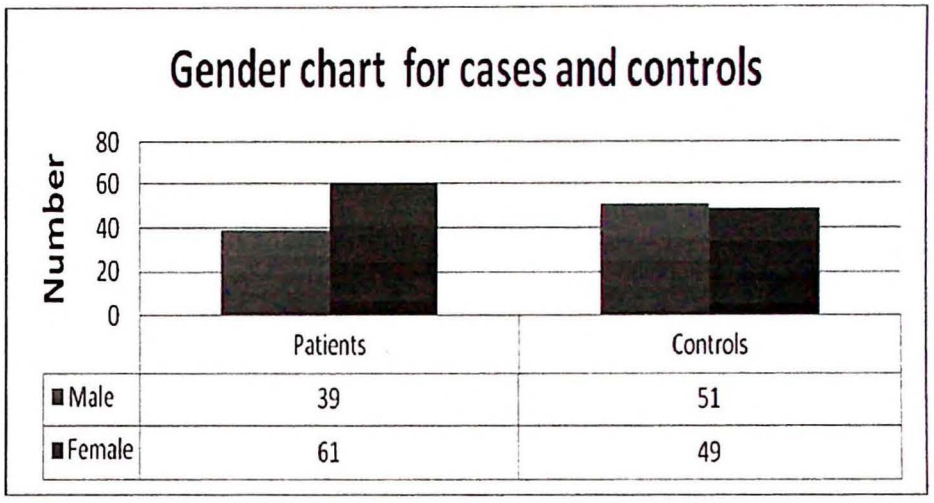
Subjects and Methodology

- The DNAs were analyzed via
  - Polymerase Chain Reaction (PCR) and
  - single-nucleotide polymorphism genotyping method.
- Allele, genotype and haplotype frequencies of these variants were compared



Flowchart of the study

Subjects and Methodology



Subjects and Methodology

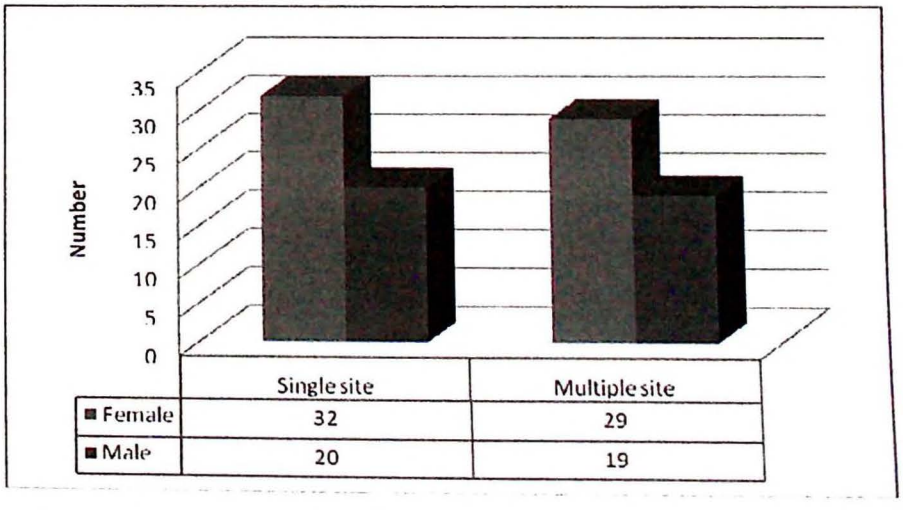


Figure : Multiple or single site of keloid among patients' group

Results

- No statistical significance was found between alleles and genotypes of *TGF*-β1 variants
  - in the case-control study in a Malay population

Results

- TGFβ1 halotypes showed a strong association with the risk of keloid formation.

Results

- The C-C halotypes of TGFβ1
  - Consisting of c.29C>T and -509T>C variants
  - showed higher frequency among keloid patients compared with the controls
    - 11% versus 2.7%
    - corrected *p*=0.037
  - showing 4.5-fold increased risk for keloid formation.

Results

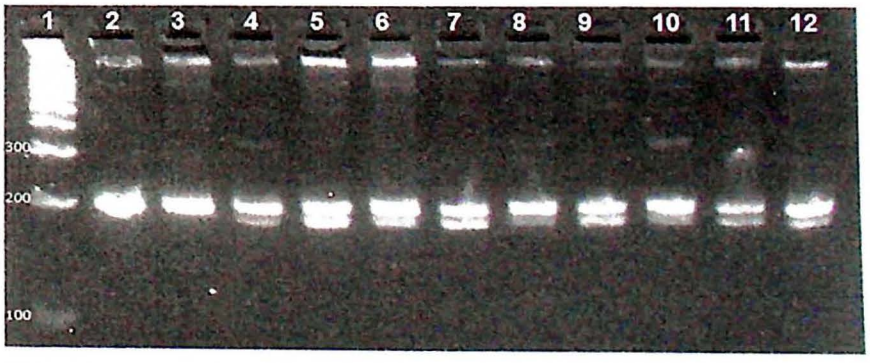


Figure : PCR amplicons consisting of c.29C>T variant of *TGFB*1 with NotI restriction enzyme on a 5% agarose gel

Lane 1: 100 bp DNA ladder
Lane 2: Undigested sample (199 bp)
Lanes 3-12: Digested PCR products

Results

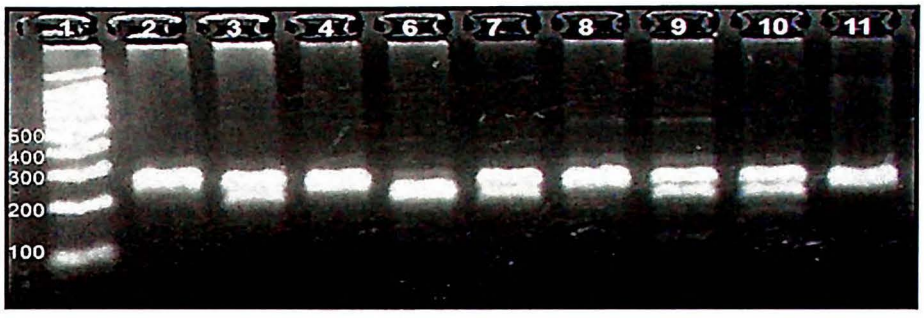


Figure: The amplicons consisting of -509 T>C variant of *TGFB*1 with Bsu36I restriction enzyme

Lane 1: 100 bp DNA ladder
Lane 2: Undigested sample with 235 bp
Lanes 3-11: Digested PCR products

Results

- The AG genotype of the *SMAD4* (c.5131A>G variant)
  - showed a statistically significant trend
  - *P-value* = 0.0573

Results

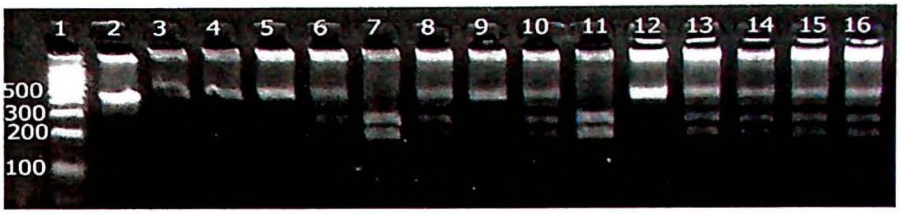


Figure : The amplicons consisting of c.5131A>G variant of *SMAD4* with TaqI restriction enzyme

Lane 1: 100 bp DNA ladder
Lane 2: Undigested PCR products with 467bp
Lanes 3-16: Digested PCR products

Results

- The C-C haplotype of *TGFβ1* variants
  - showed an increased risk when combined with both alleles of the *SMAD4* c.5131A>G variant
  - indicating possible interaction of these genes in keloid development.

Conclusion

- This is the first study

  1. documenting strong positive association between TGFβ1 variants and keloid formation

  2. providing evidence for the possible role of *SMAD4* in the development of keloid

  – in the Malay population.

Acknowledgement

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- All department staff members who contributing to the success of the study

Thank you for your attention

The Twin Tower

Kuala Lumpur

Malaysia

Contributions of TGFβ1 and SMAD4 Genes to the Etiology of Keloid Scars in the Malay Population

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Abstract:

Background: Keloid scars are complex dermal condition with genetic and environmental contributing factors. TGFβ and SMAD candidate genes, which are located in the same signaling pathway, are highly expressed in the keloid fibroblast cells. To date, only few documented reports showing relationship between TGFβ1 and keloid in Caucasian population but none on SMAD4.

Purpose: The contributions of TGFβ1 and SMAD4 in the keloid formation of Malay population were studies.

Subjects and Methodology: The DNAs were extracted from the blood samples of 100 Malay patients with keloids with another 100 healthy individuals without keloids as controls. The DNAs were analyzed via Polymerase Chain Reaction and single-nucleotide polymorphism genotyping.

Results: TGFβ1 halotypes showed a strong association with the risk of keloid formation. The CC halotypes of TGFβ1, composed of both c.29C>T and -509T>C variants, showed higher frequency among keloid patients compared with the controls (11% versus 2.7%, corrected p=0.037), showing 4.5-fold increased risk for keloid formation. The c.5131A>G variant of SMAD4 revealed a statistically significant trend (p=0.0573). Taken together, either of these variants is the most probable causative factor at the expression level or is in linkage disequilibrium with other causative variants in a complex pattern with the environmental factors, contributing to keloid formation.

Conclusion: This is the first study documenting strong positive association between TGFβ1 and SMAD4 variants and keloid formation in the Malay population.

Keywords: TGFβ1 gene, SMAD4 gene, Keloid scar, Malay