

# 1597ΔC Polymorphism and Preterm Birth in African-American Mothers

Christopher Peterson, Anthony Torres, MD, Michael Benson, Martin Kharrazi, PhD, Michelle Pearl, PhD

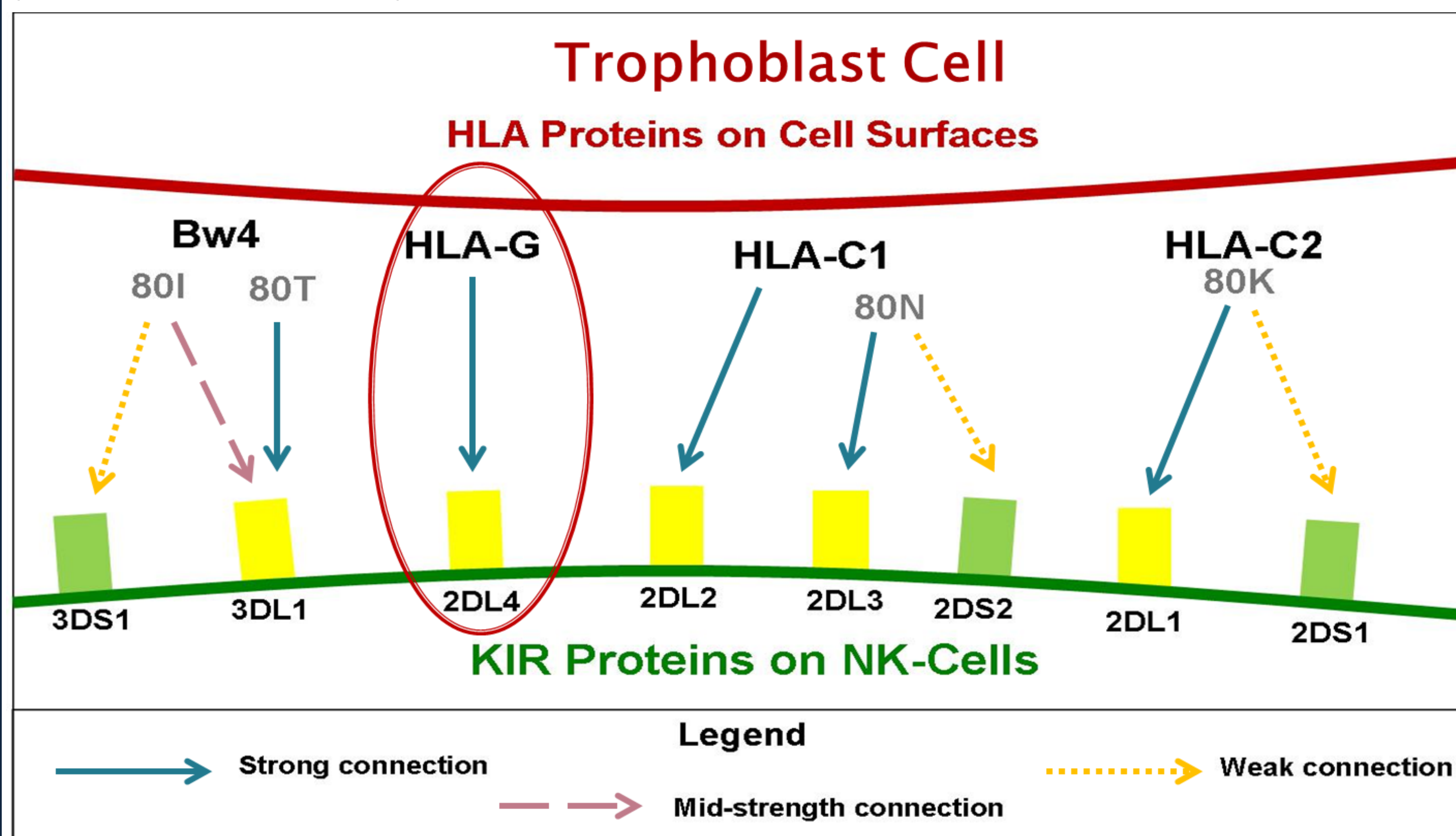
## Introduction

Natural killer cells are of particular interest during pregnancy, as they account for 70% of all lymphocytes in the placenta<sup>1</sup>. Thus, abnormalities in natural killer (NK) cells have been implicated in pre-term birth, the leading cause of infant mortality<sup>2</sup>. A suggested causative factor is a deletion at nucleotide 1597 in the HLA-G gene, which codes for the HLA-G histocompatibility antigen. HLA-G is of interest as: it is a rare “non-classical” antigen predominantly produced by fetal cells<sup>3</sup>, and HLA-G polymorphisms have been implicated in another pregnancy



**Figure 1.** Premature baby in Neonatal Intensive Care Unit

disorder: preeclampsia<sup>4</sup>. The interaction between HLA-G antigen on trophoblasts and inhibitory killer-immunoglobulin like receptor (KIR) 2DL4 on natural killer cells produces an inhibitory effect on the natural killer cell<sup>3</sup>.



**Figure 2.** Interaction of HLA-G and KIR 2DL4 between trophoblast and NK cells.

However, a deletion at nucleotide 1597 produces a frameshift mutation, rendering the HLA-G antigen unable to interact with KIR 2DL4<sup>5</sup>. It is speculated that this failed interaction might cause the mother’s immune system to allow an increased natural killer cell response to the fetus, aborting the pregnancy. Here we examine the DNA from a large population of African-American mothers to determine if this association between HLA-G 1597ΔC is causative in pre-term birth. The African-American population is of particular interest as this demographic has a uniquely high incidence of preterm birth.

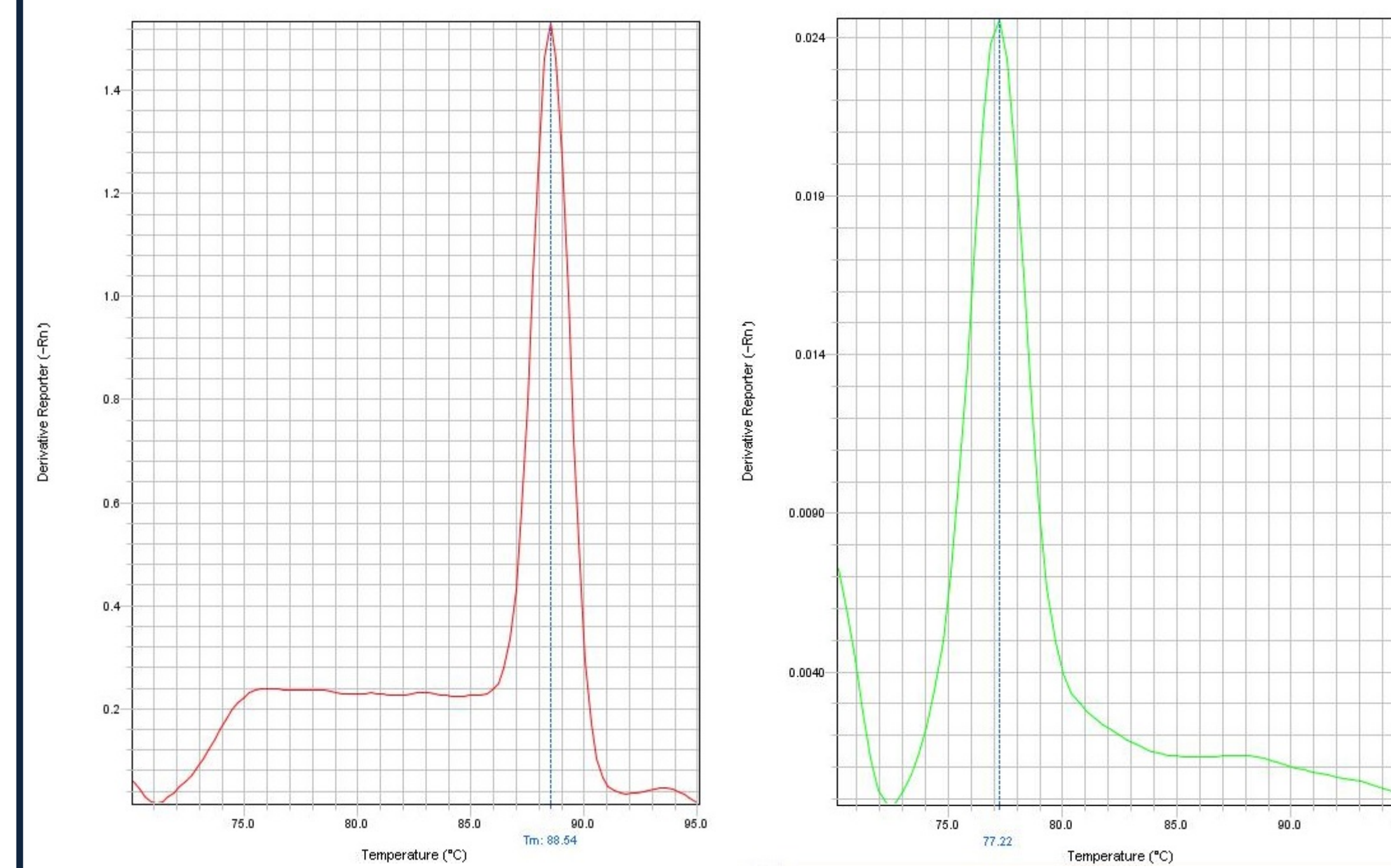
**GAACGAGGACCTGCGCTCC**

**Figure 3.** HLA-G 1597ΔC deletion site.

## Materials & Methods

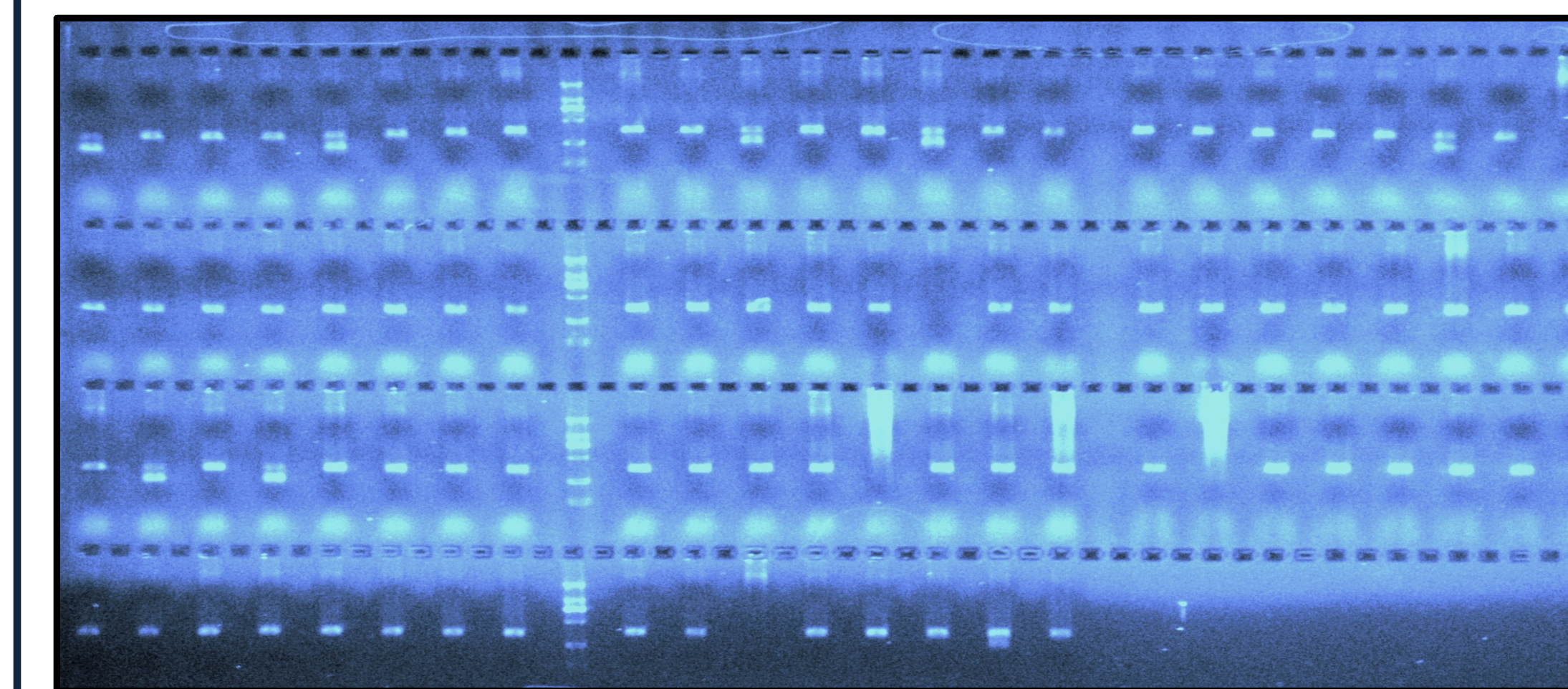
Samples are from African-American mothers who gave birth between Jan. 2000 and Apr. 2007 (California Very Preterm Birth Study)<sup>2</sup>: 343 controls, 78 preeclampsia, 330 pre-term (pre-term defined as birth at <34 weeks).

Maternal DNA was amplified using quantitative (“real-time”) PCR (qPCR), which can quantify target sequences of DNA. Each sample was run twice with primers positive (1597ΔC) and negative (HLA-G, or 1597) for the deletion. Peaks at approx. 88°C signified the deletion, and peaks at 77 °C (with absence of a positive peak) signified absence of the deletion.



**Figure 3.** qPCR peaks positive and negative for deletion, respectively. The reaction is run with a dye that causes PCR products to fluoresce. During the melt stage, fluorescence is lost as DNA strands disassociate. Loss of fluorescence occurs at a unique temperature depending on whether or not the deletion is present.

Results were confirmed using gel electrophoresis.



**Figure 4.** Maternal DNA that has been amplified with the 1597ΔC primer.

## Results

Genotype	Normal C/C	Deletion Δ/C	Deletion Δ/Δ
Individuals	630	81	2
Frequency	88.36%	11.36%	0.28%

## Conclusion

We are confirming the association between 1597ΔC and pre-term birth in this population, and a positive association would suggest that mothers possessing the deletion are at risk for delivering pre-term. As this is a double-blind study, we are unaware which genotypes correlate with pre-term birth. We are currently awaiting completion of the statistical analysis by the California Department of Health.

## References

- Yagel, Simcha. “NK Cells and Pre-Eclampsia.” *Reproductive BioMedicine Online*. 16.2 (2008):227-231.
- Kharrazi, Martin; Torres, Anthony R.; “California Very Preterm Birth Study: Design and Characteristics of the Population- and Biospecimen Bank-Based Nested Case-Control Study”. *Pediatric and Perinatal Epidemiology*. 26.3 (2012):250-26.
- Rajagopalan, S.; Long, EO. “KIR2DL4 (CD158d): An Activation Receptor for HLA-G”. *Frontiers in Immunology*. 3.258 (2012)
- Loisel, Dagan A.; Billstrand, Christine; Murray, Kathleen; Patterson, Kristen; Chaiworapongsa, Tinnakorn; Romero, Roberto and Ober, Carol. “The Maternal HLA-G 1597delta Null Mutation is Associated with Increased Risk of Pre-Eclampsia and Reduced HLA-G Expression During Pregnancy in African-American Women”. *Molecular Human Reproduction*. 19.3 (2013):144-152.
- Hunt, Joan S.; Petroff, Margaret G.; McIntire, Ramsey H., and Ober, Carole. “HLA-G and Immune Tolerance in Pregnancy”. *Federation of American Societies for Experimental Biology*. 19.7 (2005):691-693.