NK Cells & Killer
Immunoglobulin-Like Receptors: Function and Clinical Relevance

Dianne De Santis
PathWest, Royal Perth Hospital
Functions of NK cells

• Cytotoxicity against abnormal cells
  – tumour cells.
  – virus infected cells.

• Cytokine secretion – IFNg, TNF
  – Activation of macrophages, DC.
  – Influence T1/T2 balance by interaction with dendritic cells.
  – IFNg essential for Treg generation following NK:DC interaction.

• Editing/termination of immune responses
  – Kill activated DC, macrophages

=> Interface between innate and adaptive immunity.
NK Cell activation is controlled by the balance of activating and inhibitory signals.

Inhibitory signals tend to be dominant.

From Rajalingam, 2003
"Missing Self" or "Two receptor" model of NK cytotoxicity

NK cells are inhibited by self HLA class I

A. Self

B. Missing self
The KIR – HLA Lottery

KIR genes
inherited on chromosome 19

HLA alleles
inherited on chromosome 6

The KIR genes do NOT always match the HLA alleles!

We can have inhibitory KIR receptors but not the HLA ligand!
Armed NK Cells with a single inhibitory receptor for a non-self ligand are NOT ALLOWED

Inhibition encountered during development $\rightarrow$ armed NK cell

Inhibition not encountered during development $\rightarrow$ disarm
NK ALLOREACTIVITY
What is NK Alloreactivity?

Donor NK cells kill patient cells if patient cells lack the HLA alleles that are ligands for the donor’s inhibitory KIR receptors.
NK Alloreactivity: Donor must also have the Relevant Inhibitory KIR to recognise an HLA mismatch (missing self)

NK Clone 1

NK Clone 2

2DL1

C2

C1

NB. Donor’s KIR gene repertoire must include KIR2DL1

Patient

C1

C1

Killing

No Killing

Donor’s KIR gene repertoire does not include 2DL1. Unable to recognise lack of C2

=> No killing
NK ALLOREACTIVITY in HAEMATOPOIETIC STEM CELL TRANSPLANTATION
Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

Loredana Ruggeri, Marusca Capanni, Elena Urbani, Katia Perruccio, Warren D. Shlomchik, Antonella Tosti, Sabrina Posati, Daniela Rogaia, Francesco Frassoni, Franco Aversa, Massimo F. Martelli, Andrea Velardi*
Haploidentical BMTx with Potential NK Alloreactivity in the GvHD Direction Results in Less GvHD, Less Rejection and Less Relapse (AML)

<table>
<thead>
<tr>
<th></th>
<th>MATCHED</th>
<th>MISMATCHED</th>
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<tr>
<td><strong>KIR ligand incompatibility in GVH direction</strong></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Number of transplants</strong></td>
<td>58</td>
<td>34</td>
</tr>
<tr>
<td><strong>Donors displaying antirecipient NK clones</strong></td>
<td>1/58</td>
<td>34/34*</td>
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<tr>
<td><strong>Disease</strong></td>
<td></td>
<td></td>
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<tr>
<td>ALL</td>
<td>21</td>
<td>14</td>
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<tr>
<td>AML</td>
<td>37</td>
<td>20</td>
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<tr>
<td><strong>Transplantation outcomes</strong></td>
<td></td>
<td></td>
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<tr>
<td>Rejection</td>
<td>15.5%</td>
<td>0%*</td>
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<tr>
<td>Acute GVHD, ≥ grade II</td>
<td>13.7%</td>
<td>0%*</td>
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<tr>
<td>Probability of relapse at 5 years</td>
<td></td>
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<tr>
<td>ALL</td>
<td>90%</td>
<td>85%</td>
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<tr>
<td>AML</td>
<td>75%</td>
<td>0%**</td>
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\[ P \leq 0.01; \quad **P < 0.0008 \ (22) \]

EFS-AML

Benefits of Donor NK Alloreactivity

- **Donor Immune System**
  - Stem cells
  - Rejection

- **Recipient Immune System**
  - T cells
  - Graft vs Host Disease

- **Recipient Solid Organs**
  - Liver
  - Gut
  - Skin

- **Donor NK Cells**
  - Kill leukemia cells

- **APC**

- **Recipient Solid Organs**
  - LEUK
Does KIR ligand mismatching improve outcome in conventional MUD transplants?

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subgroup</th>
<th>M:L:O</th>
<th>T-dep (%)</th>
<th>ATG (%)</th>
<th>Rej</th>
<th>GVHD</th>
<th>Relapse</th>
<th>Surv</th>
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<td>–</td>
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<td>Morishima [5**]</td>
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<td>577 AML</td>
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<td>0</td>
<td>XX</td>
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<tr>
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<td></td>
<td>596 CML</td>
<td>0</td>
<td>0</td>
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<td>–</td>
<td>–</td>
<td>XXX</td>
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<tr>
<td>Morishima [5**]</td>
<td></td>
<td>617 ALL</td>
<td>0</td>
<td>0</td>
<td>XX</td>
<td>–</td>
<td>–</td>
<td>XXX</td>
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<tr>
<td>Faraq [6*]</td>
<td></td>
<td>1397:0:0</td>
<td>22⁵</td>
<td>14</td>
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<td>1180:0</td>
<td>19⁷</td>
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<td>–</td>
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<td>13254:0</td>
<td>7</td>
<td>100</td>
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<td>–</td>
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<tr>
<td>Kroger [19]</td>
<td></td>
<td>90:52:0</td>
<td>0</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>XX</td>
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<tr>
<td>Lowe [20]</td>
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<td>69:33:3</td>
<td>100⁸</td>
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<td>Miller [21]</td>
<td>EM¹</td>
<td>534:0.0</td>
<td>+/-</td>
<td>+/-</td>
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<td>–</td>
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<td>479:0.0</td>
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<td>Miller [21]</td>
<td>IM¹</td>
<td>702:0.0</td>
<td>+/-</td>
<td>+/-</td>
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<td>–</td>
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<tr>
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<td>?</td>
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<td>–</td>
<td>–</td>
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</tr>
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<td>0</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
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<td>137:0</td>
<td>0</td>
<td>100</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>0</td>
<td>100</td>
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<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Yabe [8**]</td>
<td>ATG</td>
<td>?</td>
<td>0</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Studies are arranged to group together those finding similar associations with outcome. +/- = heterogeneity with respect to this variable, ‘?’ = data not provided, X = deleterious effect found, ‘✓’ = beneficial effect found, – = no effect found at p < 0.10, one tick or cross = p < 0.05, three ticks or crosses = p < 0.01; blank entries under columns headed ‘Rej’, ‘GVHD’, ‘Relapse’ and ‘Surv’ indicate that these outcomes were not reported on in that publication.
Two Major KIR Haplotype Groups - differing in number of activating KIR

Alternate configurations of the KIR gene complex

From Martin et al, Gene, 2004
Retrospective studies of number of KIR genes (B-haplotype) present in donor and outcome in MUD transplants.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Donor</th>
<th>M:L:O</th>
<th>T-Dep (%)</th>
<th>ATG</th>
<th>Rej</th>
<th>GVHD</th>
<th>Relapse</th>
<th>Surv</th>
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<td>–</td>
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<tr>
<td>Cook [29]</td>
<td>SIB</td>
<td>112:0:0</td>
<td>?</td>
<td>?</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Cooley [9]</td>
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<td>Savari [32]</td>
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<td>100</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Verheyden [33]</td>
<td>SIBS</td>
<td>49:16:0</td>
<td>52</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>Kim [34]</td>
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<td>?</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>Kroger [19]</td>
<td>MUD</td>
<td>90:52:0</td>
<td>0</td>
<td>100</td>
<td>XX</td>
<td>XXX</td>
<td>XXX</td>
<td>XXX</td>
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<td>Triplett [36]</td>
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<td>13 + 27</td>
<td>100</td>
<td>–</td>
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<tr>
<td>Glebei [11**]</td>
<td>MUD/SIB</td>
<td>78:22:0</td>
<td>0</td>
<td>68</td>
<td>–</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>Clausen [37]</td>
<td>SIB</td>
<td>?</td>
<td>?</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Yabe [8**]</td>
<td>MUD</td>
<td>n = 187</td>
<td>0</td>
<td>6</td>
<td>XX</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Sun [23]</td>
<td>MUD</td>
<td>65:0:0</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hsu [10]</td>
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<td>?</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Studies are arranged to group together those finding similar associations with outcome.

*?* data not provided, *X*: deleterious effect found, *✓*: beneficial effect found, –: no effect found at \( p < 0.10 \), one tick or cross = \( p < 0.1 \), two ticks or crosses = \( p < 0.05 \), three ticks or crosses = \( p < 0.01 \); blank cells for rejection, GVHD, relapse and survival indicate that these outcomes were not reported on.
KIR B-haplotype donors result in improved survival in multicentre study of AML

More KIR genes (B/x)  
$p=0.007$

Fewer KIR genes (A/A)

Cooley et al  
Blood, 2009
Donors with more KIR genes have beneficial effect on relapse and survival in AML

Cooley et al, Blood, 2010
Donors with fewer KIR genes result in improved survival

Kroger et al, Transplantation, 2006
Donors with fewer KIR genes protect against relapse.

Kroger et al, Transplantation, 2006
How can we explain such contrasting results in different transplant centres?

The size effect of donor KIR genotype appears at least as big as mismatching one HLA locus. Therefore we would like to use it for donor selection but which effect will we get?

Could there be something in the transplant protocol that interacts with KIR genotype?
Why the Different Effects?

- **Heterogeneous disease groups**
  - Myeloid vs lymphocytic leukemias, other diseases

- **Stem Cell Source**
  - T replete vs T depleted
  - Use of ATG
  - BM vs Peripheral Stem cells

- **Conditioning, GVHD prophylaxis**

- **HLA Matching**

- **Limited Power**
  - Univariate vs multivariate analysis
Could differences in transplant protocol be responsible for the different effects??

Year of 133 MUD TransplantsPerformed at Royal Perth Hospital

Diagnoses of 133 MUD Transplants(RPH)
Transplant Variables Relevant to NK Cells

- **Cytomegalovirus (CMV) status & CMV Prophylaxis**
  - NK cells (KIR) are important for control of CMV reactivation.
  - CMV+ patients have worse outcomes.

- **Peripheral blood or Bone Marrow**
  - PBSC grafts have more lymphocytes (NK cells)

- **Conditioning Drugs and Total Body Irradiation (TBI)**
  - TBI and cytotoxic drugs up-regulate stress ligands on leukaemia cells for NK cell receptors.
  - Increased cytotoxicity towards tumor cells.
TBI may have a weak interaction with KIR2DS2 -of the kind we are looking for.

---

**TBI + Transplants**

Survival Functions

**2DS2+**

**2DS2-**

$p=0.05$

**TBI- Transplants**

Survival Functions

**2DS2+**

**2DS2-**

$p=0.27$
Cyclophosphamide has a strong interaction with KIR2DS2 -of the kind we are looking for.

Cy+ Transplants

Cy- Transplants

Survival Functions

Survival Functions

2DS2+

2DS2-

p=0.003

p=0.032
Absence of KIR3DS1 is beneficial in PBSC transplants

PBSC

Marrow

Survival Functions

CumSurvival

SURVIVED

1.0
0.8
0.6
0.4
0.2
0.0

3DS1-
p=0.013
3DS1+

Survival Functions

CumSurvival

SURVIVED

1.0
0.8
0.6
0.4
0.2
0.0

Absence of KIR2DS5 is beneficial in CMV Neg transplants

CMV- Transplants

CMV+ Transplants

Survival Functions

2DS5-
p=0.001

2DS5+
### Telomeric KIR Genes Interact with Source and CMV Status

### Centromeric KIR Interact with Conditioning Agents

<table>
<thead>
<tr>
<th>KIR Genes</th>
<th>Peri. Blood</th>
<th>Bone Marrow</th>
<th>Tx CMV-1</th>
<th>Tx CMV+2</th>
<th>Cy-3</th>
<th>Cy+4</th>
<th>Bu-5</th>
<th>Bu+6</th>
<th>Flu-7</th>
<th>Flu+8</th>
<th>Mel-9</th>
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<th>TBI-11</th>
<th>TBI+12</th>
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<tr>
<td>2DL2</td>
<td>0.944</td>
<td>0.751</td>
<td>0.837</td>
<td>0.777</td>
<td>0.032</td>
<td>0.002</td>
<td>0.287</td>
<td>0.577</td>
<td>0.196</td>
<td>0.036</td>
<td>0.020</td>
<td>0.064</td>
<td>0.151</td>
<td>0.028</td>
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<td>2DL5</td>
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<td>0.386</td>
<td>0.621</td>
<td>0.123</td>
<td>0.605</td>
<td>0.489</td>
<td>0.659</td>
<td>0.859</td>
<td>0.238</td>
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<td>0.179</td>
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<td>0.943</td>
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<td>0.028</td>
<td>0.863</td>
<td>0.005</td>
<td>0.592</td>
<td>0.088</td>
<td>0.968</td>
<td>0.450</td>
<td>0.294</td>
<td>0.373</td>
<td>0.467</td>
<td>0.880</td>
<td>0.102</td>
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<td>2DS2</td>
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<td>0.690</td>
<td>0.837</td>
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<td>0.032</td>
<td>0.002</td>
<td>0.309</td>
<td>0.577</td>
<td>0.198</td>
<td>0.038</td>
<td>0.024</td>
<td>0.064</td>
<td>0.151</td>
<td>0.034</td>
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<td>0.602</td>
<td>0.170</td>
<td>0.069</td>
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<td>0.417</td>
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<td>0.184</td>
<td>0.281</td>
<td>0.204</td>
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<td>0.674</td>
<td>0.001</td>
<td>0.937</td>
<td>0.328</td>
<td>0.120</td>
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<td>0.485</td>
<td>0.063</td>
<td>0.878</td>
<td>0.147</td>
<td>0.318</td>
<td>0.481</td>
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<td>0.003</td>
<td>0.952</td>
<td>0.049</td>
<td>0.718</td>
<td>0.372</td>
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<td>0.284</td>
<td>0.257</td>
<td>0.454</td>
<td>0.083</td>
<td>0.096</td>
<td>0.593</td>
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</table>

Table 15: P-values of all the conditioning variables with individual KIR genes on survival rate.

1 Transplants with both patient and donor CMV negative. 2 Transplants with at 1 patient or donor CMV positive. 3 Transplant regimens with no cytosphosphamide. 4 Transplants with cytosphosphamide. 5 Transplant regimens with no busulphan. 6 Transplant regimens with busulphan. 7 Transplant regimens with no fludarabine. 8 Transplant regimens with fludarabine. 9 Transplant regimens with no melphalan. 10 Transplant regimens with melphalan. 11 Transplant regimens with no total body irradiation. 12 Transplant regimens with total body irradiation.
CONCLUSIONS

• Interactions between KIR genes and other transplant variables may explain the conflicting reports of the effect of donor KIR genotype.

• Cyclophosphamide is used less now than previously and may be the main factor that interacts with the centromeric B haplotype genes (2DS2/2DL2)

• Given the history of this field, these findings need confirmation!!
KIR IN DISEASE

PRE-ECLAMPSIA
&
RECURRENT SPONTANEOUS ABORTION
Decidua NK Cells in pregnancy

-NK cells constitute 50-90% of lymphocytes in decidua
-Express highest amount of CD56 and are skewed towards cytokine production
-On embryo implantation & placentation, uterine NK cells cooperate with extravillous trophoblasts to remodel the spiral arteries
-Extravillous trophoblasts express the MHC class I molecules, HLA-C, E and G, all good ligands for NK cell receptors
-Paternal HLA-C is expressed on trophoblast cell surface
Combinations of Maternal KIR and Fetal HLA-C Genes Influence the Risk of Preeclampsia and Reproductive Success

Susan E. Hibi,1 James J. Walker,2 Kevin M. O’Shaughnessy,3 Christopher W.G. Redman,4 Mary Carrington,5 John Trowsdale,1 and Ashley Moffett1

Association of maternal killer-cell immunoglobulin-like receptors and parental HLA-C genotypes with recurrent miscarriage

S.E. Hibi1, L. Regan2, W. Lo2, L. Farrell1, M. Carrington3 and A. Moffett1,4

1Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK; 2Department of Obstetrics and Gynaecology, St Mary’s Hospital Medical School, Norfolk Place, London W2 1PG, UK; 3Laboratory of Genomic Diversity, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD 21702, USA
Pre-eclampsia and recurrent miscarriage are attributed to inadequate trophoblast invasion could the interaction between uterine NK cells and extravillous trophoblast be important in such diseases
Homozygosity for the KIR “A Haplotype” in the mother predisposes to PE, risk is further increased when foetus expresses C2 epitope.
The risk of pre-eclampsia was greater when the fetus had more copies of HLA-C2 than mother and disease was more prevalent when the fetus expressed paternal HLA-C2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ORA</th>
<th>P</th>
<th>n (affected/controls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effect of relative dose of maternal and fetal C2 genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fetus had fewer C2 genes than the mother</td>
<td>0.97</td>
<td>1.00</td>
<td>177/85</td>
</tr>
<tr>
<td>Fetus had the same number of C2 genes</td>
<td>1.43</td>
<td>0.06</td>
<td>364/233</td>
</tr>
<tr>
<td>Fetus had more C2 genes than the mother</td>
<td>2.09 (1.24–3.51)</td>
<td>0.007</td>
<td>188/105</td>
</tr>
<tr>
<td>Effect of origin of fetal C2 genes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal origin</td>
<td>2.02 (1.14–3.58)</td>
<td>0.022</td>
<td>135/90</td>
</tr>
<tr>
<td>Maternal origin</td>
<td>1.11</td>
<td>0.90</td>
<td>91/61</td>
</tr>
</tbody>
</table>

A Where shown, values in parentheses denote 95% CI. B See Figure 5A for groupings. ORs and P values were calculated for the relative frequency of the maternal KIR AA genotype in control and affected pregnancies. C See Figure 5B for groupings.
The telomeric-B of the KIR B haplotype protect against disorders of pregnancy, particularly when the fetus has an HLA-C2 gene.

<table>
<thead>
<tr>
<th>Maternal KIR B regions present(^4)</th>
<th>KIR genotype frequencies (%) in all controls and affected cases</th>
<th>Maternal KIR frequencies (%) in pregnancies with fetal C2</th>
<th>Maternal KIR frequencies (%) only in pregnancies with fetal C1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n=592)</td>
<td>Affected (n=975)</td>
<td>Controls (n=235)</td>
</tr>
<tr>
<td>None (KIR AA)</td>
<td>27.5</td>
<td>36.9(^c)</td>
<td>17.0</td>
</tr>
<tr>
<td>Cen-B alone</td>
<td>27.4</td>
<td>30.1</td>
<td>14.2</td>
</tr>
<tr>
<td>Tel-B alone</td>
<td>19.3</td>
<td>14.6(^o)</td>
<td>11.1</td>
</tr>
<tr>
<td>Cen-B plus Tel-B</td>
<td>25.8</td>
<td>18.5(^e)</td>
<td>13.2</td>
</tr>
<tr>
<td>All with Tel-B(^b)</td>
<td>45.1</td>
<td>33.0(^f)</td>
<td>24.3</td>
</tr>
<tr>
<td>Trend test</td>
<td>(P &lt; 0.001)</td>
<td></td>
<td>(P = 0.002)</td>
</tr>
</tbody>
</table>

\(^4\)All affected women (preeclampsia, FGR, and RM) were grouped according to whether they had any KIR B haplotype genes in the centromeric (Cen-B) and/or telomeric (Tel-B) region. The frequency of these KIR genotypes was compared in affected and control pregnancies. \(^{ib}\)Includes both Tel-B alone and Cen-B plus Tel-B groups. (Separate results are shown for each pregnancy disorder in Supplemental Figure 6). The trend from no KIR B genes (AA genotype) to possession of Tel-B genes was highly significant \((P < 0.001)\). Preeclampsia and FGR pregnancies were divided into those with a C2 carrier fetus and those with a C1/C1 fetus. Reduced group sizes were due to omission of the women with RM plus some patients from the affected cohorts in which the baby was not available. The trend from AA genotype to presence of Tel-B region KIR was significant \((P = 0.002)\) only when there was a C2 allele present in the fetus. \(^{oc}\)\(^{P} = 1.3 \times 10^{-4}\), OR 1.54 (1.23–1.92). \(^{dp}\)\(^{P} = 0.019\), OR 0.71 (0.55–0.94). \(^{fp}\)\(^{P} = 7.4 \times 10^{-4}\), OR 0.65 (0.51–0.83). \(^{gp}\)\(^{P} = 1.7 \times 10^{-4}\), OR 0.60 (0.49–0.74). \(^{op}\)\(^{P} = 0.01\), OR 1.49 (1.10–2.01). \(^{hp}\)\(^{P} = 0.039\), OR 0.74 (0.56–0.97).
Recurrent miscarriage was found to be associated with the KIR “AA” Haplotype in the mother and an increase frequency of HLA-C2 in RM couples.

- The increased frequency of HLA-C2 in RM couples predicts an increase of HLA-C2 in the foetus.
THANK YOU