

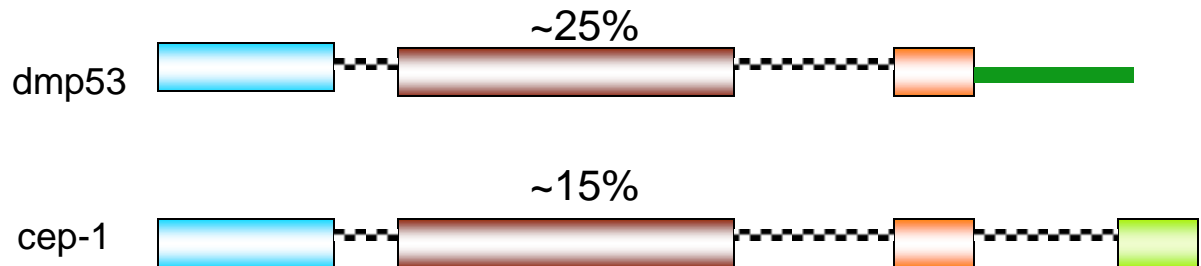
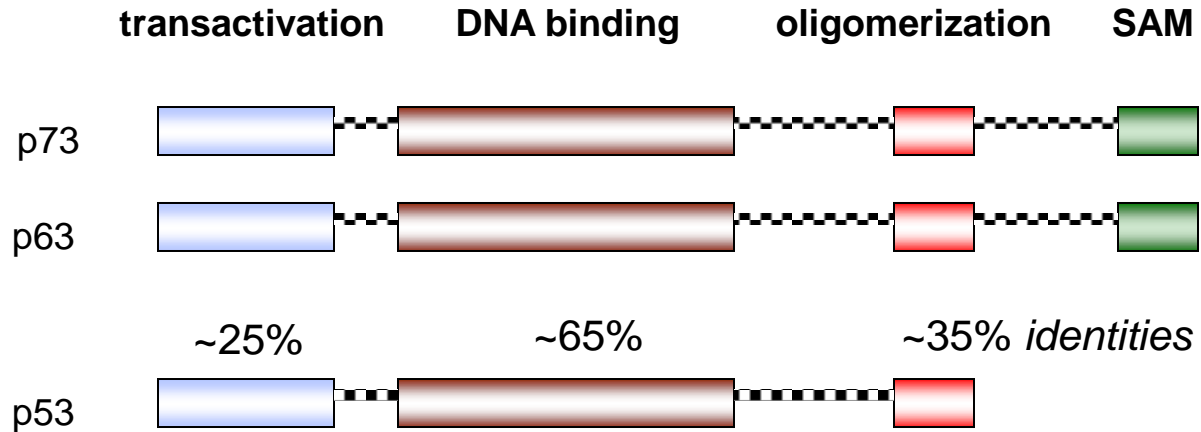
starlet sea anemone  
*Nematostella vectensis*



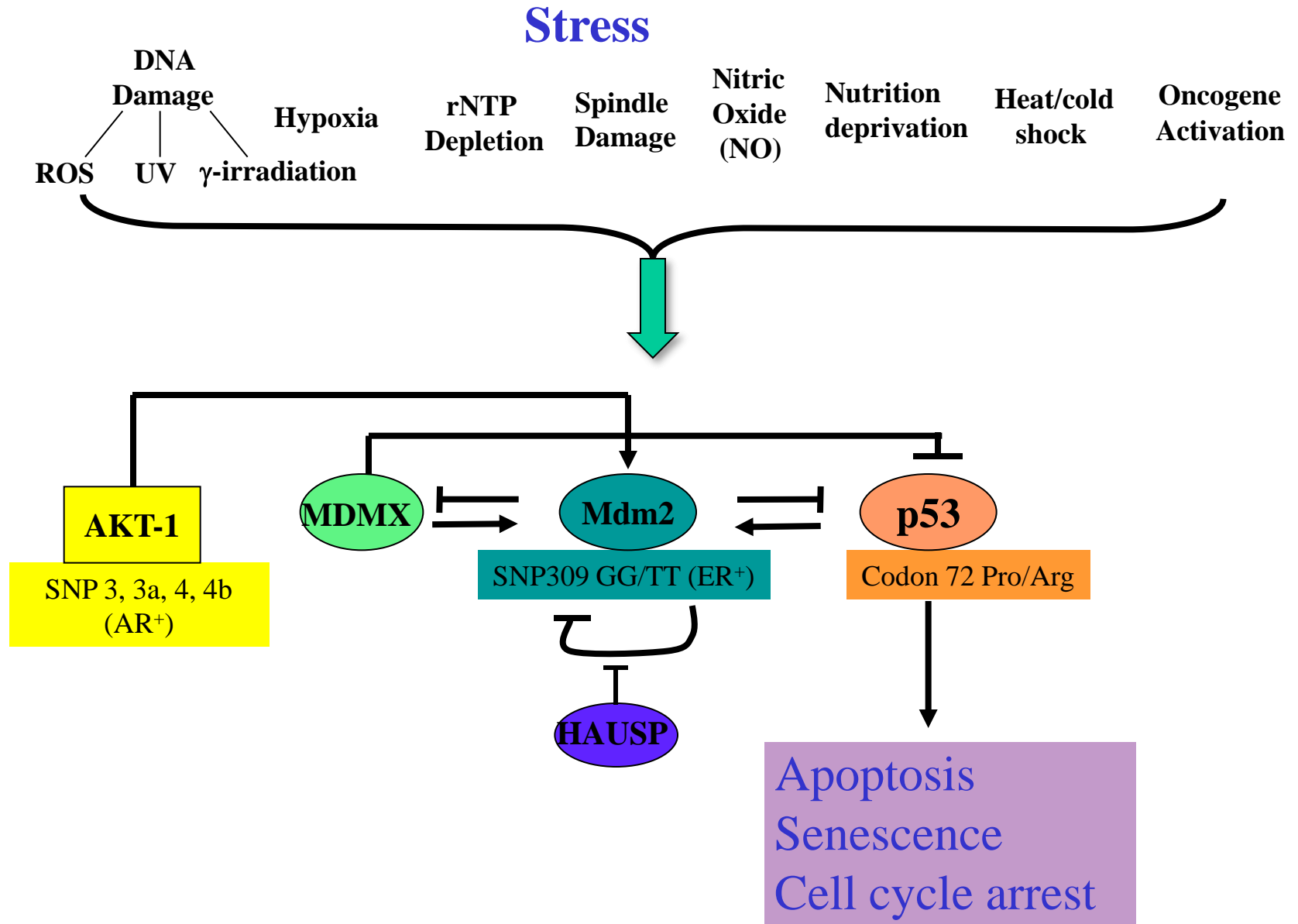
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# p53 FAMILY PROTEIN STRUCTURE

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# SNPs in the p53 pathway



Summary of gene diversity for p53 family in human populations from HapMap Phase 3.

Gene symbol	Population <sup>a</sup>	Sample size ( <i>N</i> ) <sub><i>b</i></sub>	SNP number	Haplotype number	Haplotype diversity <sup>c</sup>
<i>TP53</i>	African (YRI)	113	7	15	0.843
	Caucasian (CEU)	113	7	10	0.489
	Chinese (CHB)	84	9	11	0.748
<i>TP63</i>	African (YRI)	113	152	219	1.000
	Caucasian (CEU)	113	160	215	0.999
	Chinese (CHB)	84	147	168	1.000
<i>TP73</i>	African (YRI)	113	45	169	0.996
	Caucasian (CEU)	113	44	153	0.993
	Chinese (CHB)	84	45	142	0.997

**C57Bl/6**

Genotype for p53		Litter size		Fertility rate	Sex ratio		♀
Male	Female	Average±SE	n		Male:Female	n	Average
+/+	+/+	6.71 ±0.43	14	100%	0.51:0.49	107	6.54
-/-	+/+	6.41 ±0.61	11	100%	0.53:0.47	116	(100%)
+/-	+/-	4.79 ±0.61	19	79%	0.57:0.43	153	5.05
-/-	+/-	5.11 ±0.21	89	92%	0.62:0.38	856	(77%)
-/-	-/-	<b>0.69 ±0.16</b>	<b>69</b>	<b>27%</b>	<b>0.85:0.15</b>	<b>65</b>	<b>0.77</b>
+/+	-/-	<b>2.18 ±0.83</b>	<b>11</b>	<b>36%</b>	<b>0.43:0.57</b>	<b>14</b>	<b>(11.8%)</b>
+/-	-/-	<b>1.42 ±0.55</b>	<b>12</b>	<b>33%</b>	<b>NA</b>	<b>NA</b>	

**129**

Genotype for p53		Litter size		Fertility rate	Sex ratio		♀
Male	Female	Average±SE	n		Male:Female	n	Average
+/+	+/+	5.41 ±0.35	22	95%	0.5:0.5	244	5.38
-/-	+/+	5.5 ±0.5	6	100%	0.53:0.47	16	(100%)
+/-	+/-	5.21 ±0.64	8	100%	0.55:0.45	140	4.88
-/-	+/-	4.8 ±0.18	32	84%	0.54:0.46	357	(90.7%)
-/-	-/-	<b>2.35 ±0.24</b>	<b>90</b>	<b>58%</b>	<b>0.57:0.43</b>	<b>321</b>	<b>2.35</b>
+/+	-/-	<b>2.75 ±1.6</b>	<b>4</b>	<b>50%</b>	<b>0.45:0.55</b>	<b>11</b>	<b>(43.7%)</b>
+/-	-/-	<b>2</b>	<b>1</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	

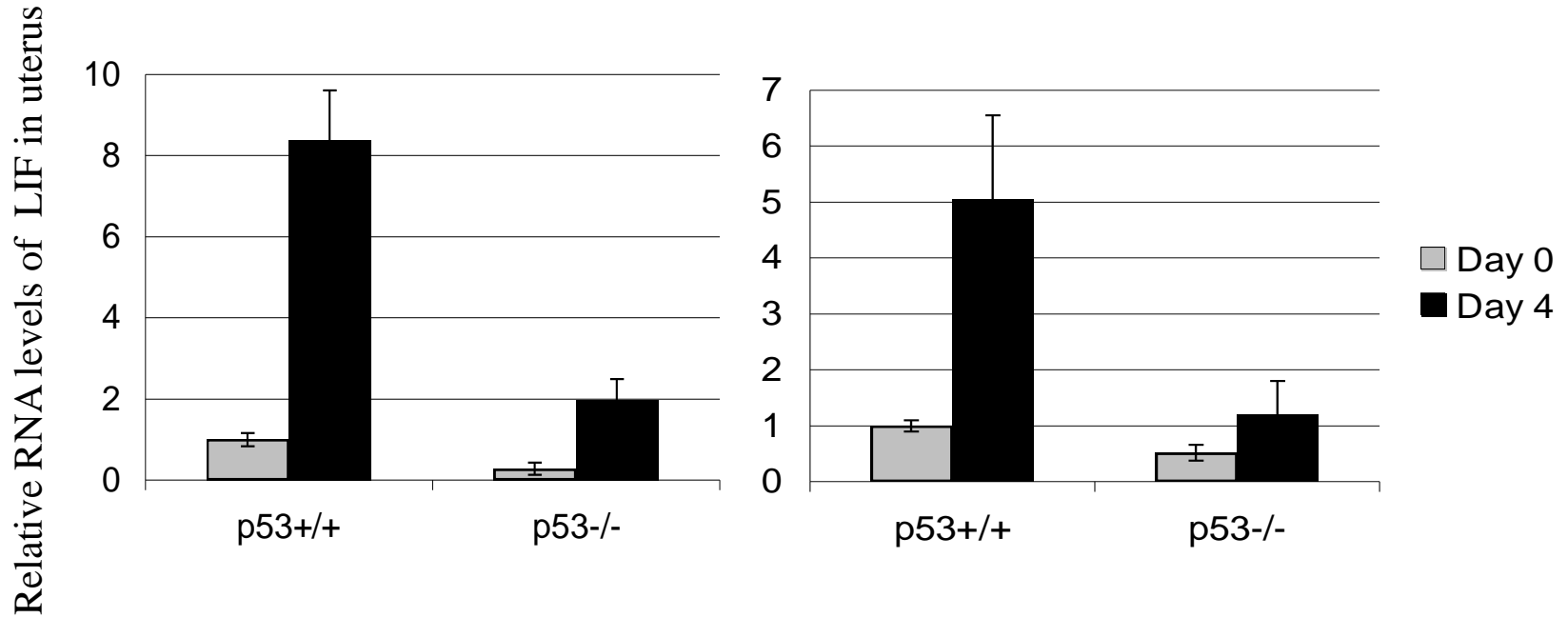
**C57Bl/6 × 129**  
 (Sire ♂) (Dam ♀)

Genotype for p53		Litter size		Fertility rate	Sex ratio	
Male	Female	Average±SE	n		Male:Female	n
-/-	-/-	2.62 ±0.84	8	63%	0.59:0.41	22

# Decreased LIF levels in uterus in p53 null mice

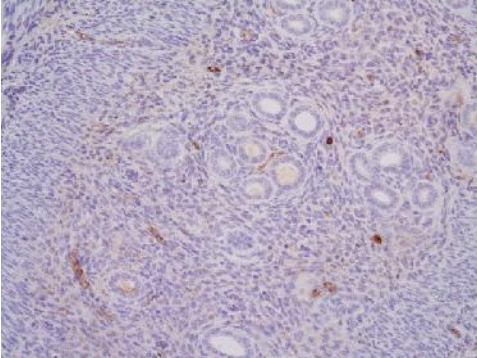
C57BL/6J

129SV<sup>sl</sup>

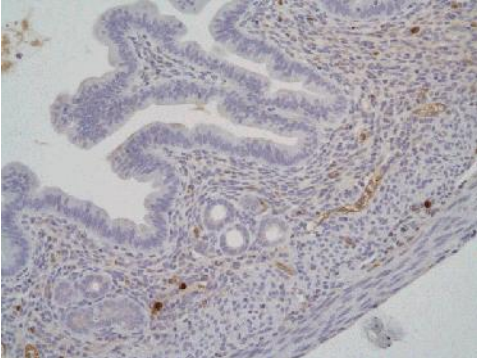


# Increased p53 protein levels in mouse uterine endometrium during pregnancy

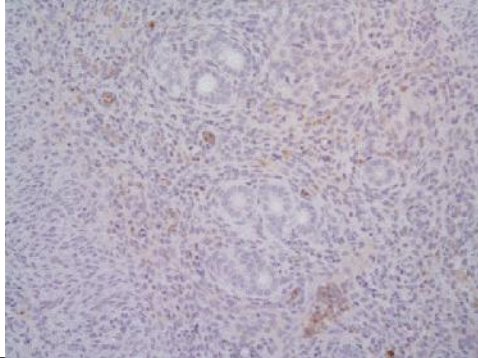
D0



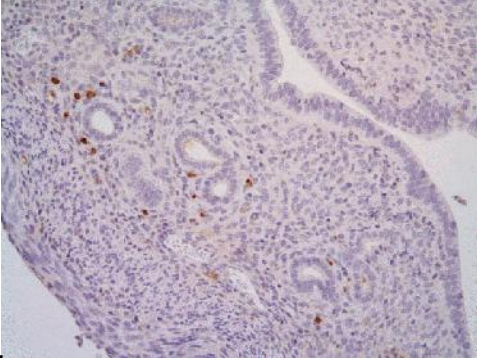
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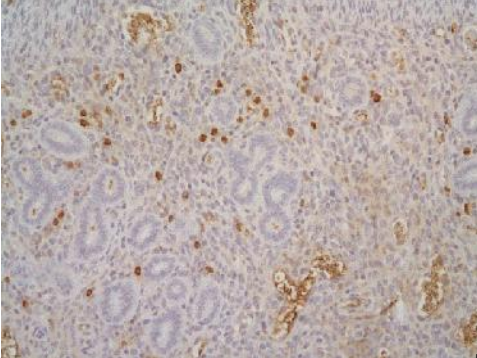
D2



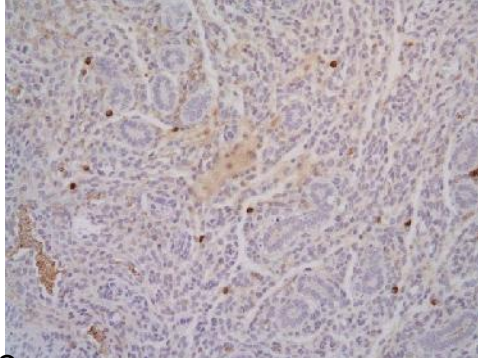
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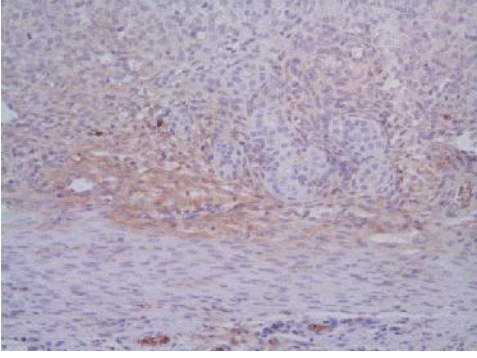
D4



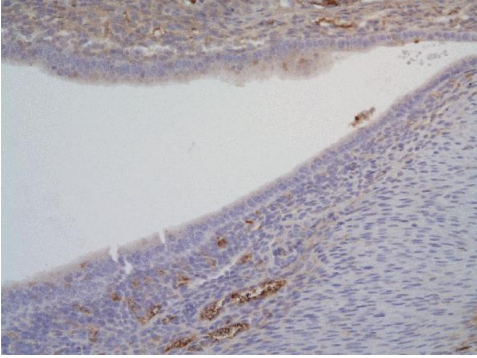
D5



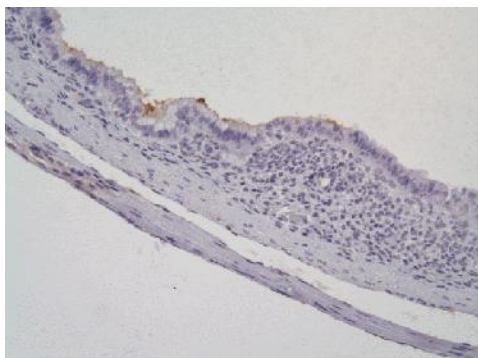
D7



D9



D13



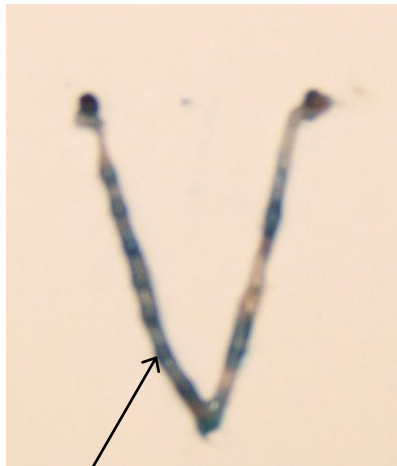
# The impaired implantation in p53 null mice and its restoration by LIF injection

Day 5

p53+/+

p53-/-

p53-/- +LIF injection



Implantation sites

Genotype of C57BL/6J mice		LIF injection	Implantation sites (Average±SE)	Number of recovered blastocysts (Average±SE)	n
Male	Female				
+/+	+/+	-	8.4±0.5	0	5
-/-	-/-	-	2.7±0.8	3.2 ±0.6	6
-/-	-/-	+	7±0.8	0.6±0.6	3

# P53 Tumor Suppressor Gene Polymorphism is Associated with Recurrent Implantation Failure

C. Kay, R.S. Jeyendran, and C.B. Coulam

p53 codon 72: Proline frequency enhanced in 205 women with recurrent pregnancy loss in an IVF clinic due to recurrent implantation failure

$$p = 0.003$$

## Allelic frequencies of the SNPs in the p53 pathway in IVF patients

	Genotype of SNPs	Control n (%)	IVF patients			
			<35 yrs		≥ 35 yrs	
			n (%)	p	n (%)	p
<b>p53 codon 72</b>	ARG	1812 (77.3)	215 (64.8)	1.8E-06	149 (70.3)	0.03
	Pro	532 (22.7)	117 (35.2)		63 (29.7)	
<b>LIF</b>	T	1082 (71.4)	210 (63.3)	0.008	146 (68.9)	0.43
	G	434 (28.6)	122 (36.7)		66 (31.1)	
<b>Mdm2 SNP309</b>	T	1639 (62.7)	184 (55.7)	0.03	123 (58.0)	0.35
	G	977 (37.3)	148 (44.3)		89 (42.0)	
<b>Mdm4</b>	C	1727 (68.4)	205 (61.7)	0.05	125 (59.0)	0.02
	T	799 (31.6)	127 (38.3)		87 (41.0)	
<b>Hausp</b>	G	137 (68.5)	186 (56.0)	0.004	138 (65.1)	0.46
	A	63 (31.5)	146 (44.0)		74 (34.9)	

# Roles of p63 and p73 in the Female Germ Line

1. TA p63 is required for female egg production and plays a role in Meiosis. Shu et al, 2006.
2. TA p63 responsible for oocyte death after DNA damage. Gonfloni et al, 2009.
3. TA p73 Required for primordial and primary oocytes. Tomasini et al, 2008.

4. TA p63, TA p73: Transcribed Genes  
BRCA 1, 2, mre-11, RAD 51,

5. TA p73 acts at G-2 and spindle check  
points, Tomasini et al, 2009.

6. TA p63 -I- mice - sterile.

TA p73 -I- mice - older mice less eggs.

Tomisini et al, 2008.

## Allelic frequencies of the SNPs in p63 and p73 in IVF patients

### p63

rs17506395

	Control % (n)	Young % (n)		Older % (n)		Total % (n)	
G	29.1(113)	21.7 (72)	p=0.02	20.8 (72)	p=0.01	21.2 (144)	p=0.004
T	70.9 (275)	78.3 (260)		79.2 (274)		78.8 (534)	

### p73

rs4648551

	Control % (n)	Young % (n)		Older % (n)		Total % (n)	
A	43.1(168)	41.6 (138)	p=0.70	33.5 (116)	p=0.004	37.4 (254)	p=0.04
G	56.9 (222)	58.4 (194)		66.5 (230)		62.6 (424)	

# Women in the IVF Clinic SNPs in genes that predispose to Aneuploidy

Gene	SNP	p-value
Top 63	rs1756395	0.007
Top 3B	rs9610750	0.009

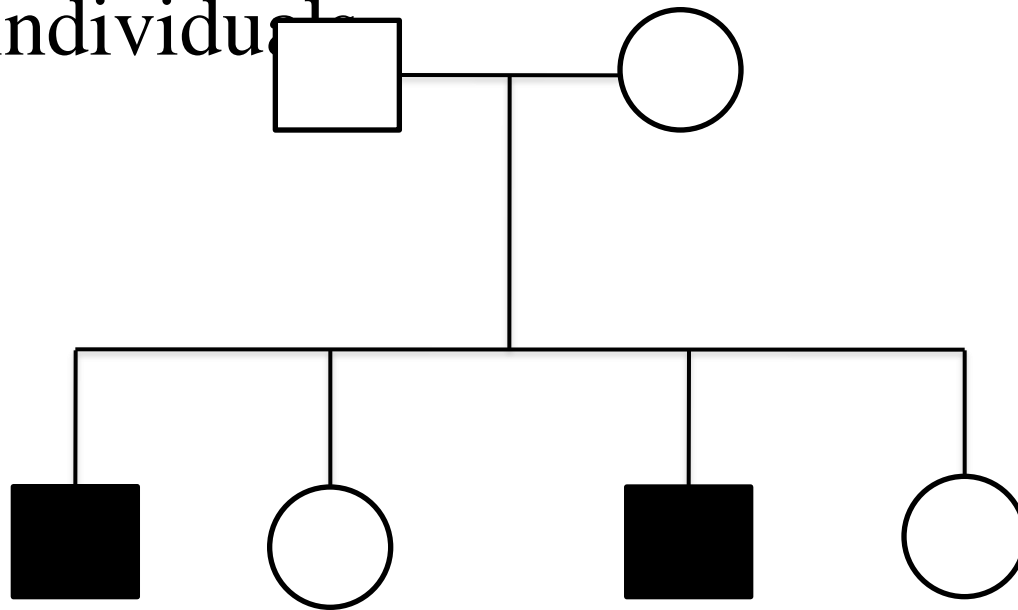
**84 patients, 4 embryos tested  
under 35 years of age**

# Germ line Fidelity

- Determined by proteins that effect DNA repair, replication, cell cycle regulation, and chromosome segregation.
- Mutations that affect these functions can reduce the fidelity of genetic transmission.
- The phenotype is genomic instability.
- We look at the parents, focusing on the p53 family of genes.

# Autism Genetic Resource Exchange (AGRE)

- 900 families (850 multiplex and 50 simplex)
- 5000 individuals



# Case-Control Analysis

- Look for **specific mutations** that are responsible for disease.
- CNVs
- SNPs
- Polygenic effects

- SNP chip data for the family.
- Two SNP chips used in AGRE scan about 500K SNPs
  - 1) Affymetrix 5.0 (~3000 individuals from 700 families)
  - 2) Illumina 550K (~4500 individuals from 900 families)

# AGRE Families

Tp63, rs4687090 - Frequency

	Mothers	Fathers
<b>MAF</b>	<b>44.3%</b>	<b>39.2%</b>
<b>#</b>	<b>954</b>	<b>1204</b>
<b>p-value</b>		<b>0.03</b>

## Recombination Events

	Mothers			Fathers		
	AA	Aa	aa	AA	Aa	aa
	<b>42.8</b>	<b>43.8</b>	<b>44.5</b>	<b>26.8</b>	<b>26.5</b>	<b>27.6</b>
<b>p-value</b>	<b>0.2</b>	<b>0.05</b>		<b>0.7</b>	<b>0.1</b>	

# AGRE Families

Tp73, rs6695878 - Frequency

	Mothers	Fathers	Control
<b>MAF</b>	<b>5.7%</b>	<b>3.73%</b>	<b>3.8%</b>
<b>#</b>	<b>(942)</b>	<b>(884)</b>	<b>(2915)</b>
<b>p-value</b>		<b>0.03</b>	<b>0.004</b>

## Recombination Events

	Mothers			Fathers		
	AA	Aa	aa	AA	Aa	aa
	<b>43.4</b>	<b>45.4</b>	<b>59</b>	<b>26.8</b>	<b>26.3</b>	<b>—</b>
<b>p-value</b>		<b>0.09</b>	<b>0.02</b>			<b>0.70</b>

# AGRE Families

H2AFY2, rs3750770, Frequency

	Mothers	Fathers	Control
<b>MAF</b>	<b>8.5%</b>	<b>9.9%</b>	<b>7.3%</b>
<b>#</b>	<b>(948)</b>	<b>(912)</b>	<b>(2915)</b>
<b>p-value</b>		<b>0.1</b>	<b>0.005</b>

## Recombination Events

	Mothers			Fathers		
	AA	Aa	aa	AA	Aa	aa
<b>events</b>	<b>43.5</b>	<b>42.6</b>	<b>56.4</b>	<b>26.5</b>	<b>26.9</b>	<b>34.2</b>
<b>p-value</b>			<b>0.0007</b>			<b><math>3.4 \times 10^{-6}</math></b>

# De novo Copy Number Variation Deletions (10 SNPs)

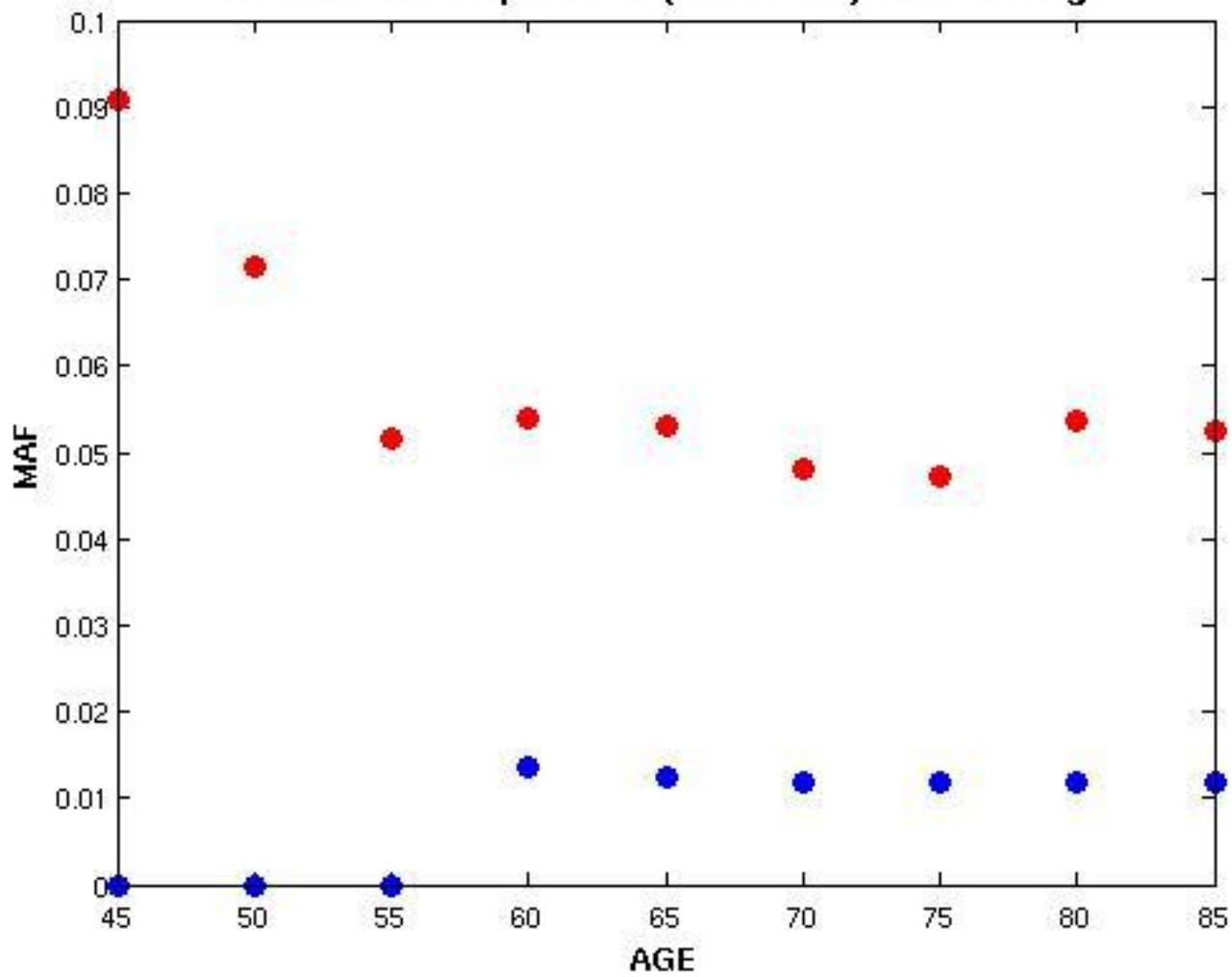
<b>AGRE Offspring</b>	<b># Deletions</b>
<b>Tp 73</b>	
<b>Minor Allele (1,2)</b>	<b>7</b>
<b>Major Allele (2)</b>	<b>3</b>
<b>Control Offspring</b>	<b>1</b>

# Simons Collection – Gene Based Test for association with de novo CNVs

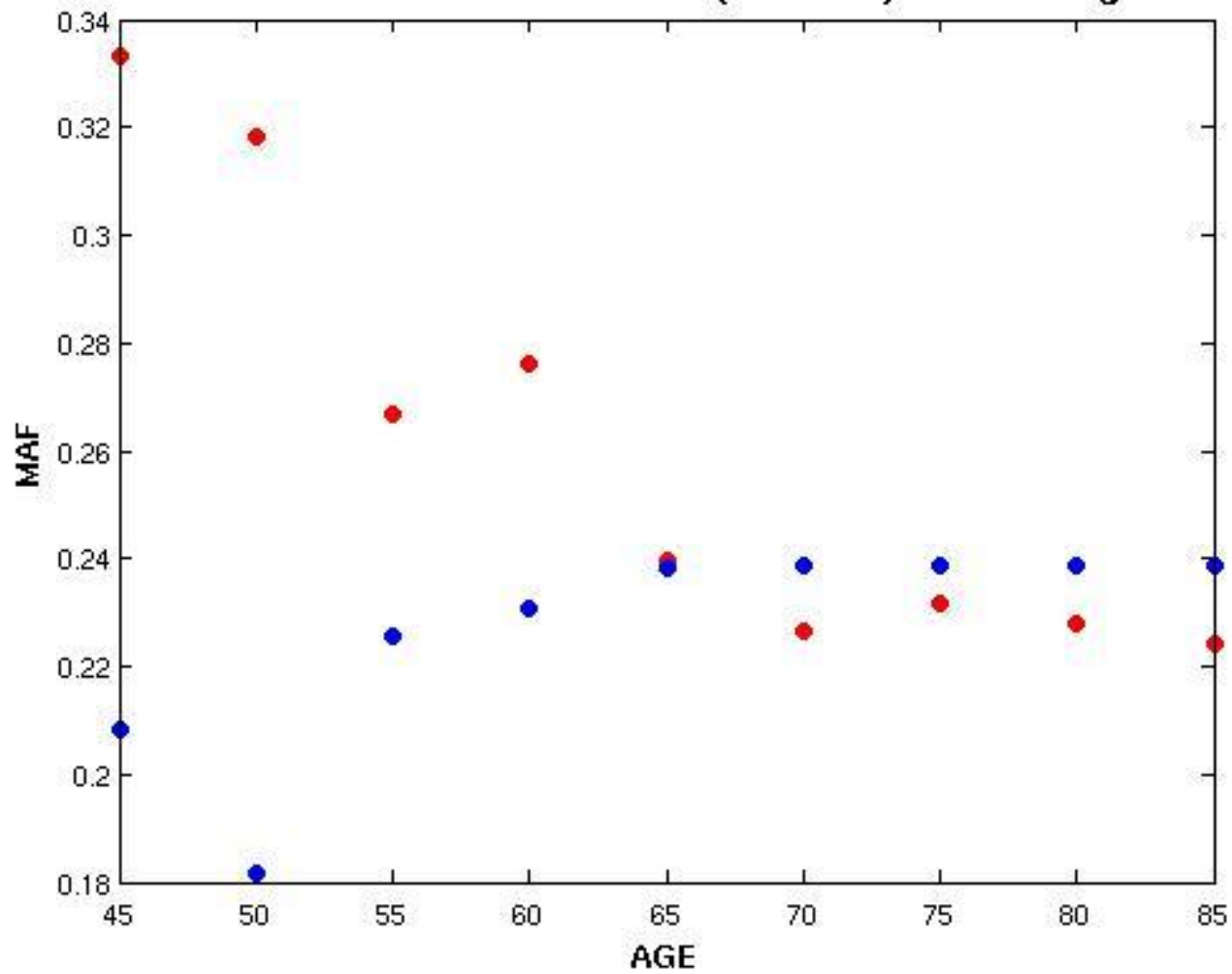
- Consider parents in families with a de novo mutation as cases and the rest of the parents as controls.
- Find the subset of SNPs with p-value  $< 0.1$  for different allele frequencies in cases vs controls in genes **TP63** and **TP73**.
- From this subset, find the set of independent (unlinked) SNPs with  $r^2 < 0.5$ .
- Find the average p-value of this independent subset of SNPs. This is our test statistic.
- Find the same average p-value after permuting the case control labels for 10000 permutations.
- The final p-value is the fraction of times the permutations yield a test statistic that is better than the original labels.

	# SNPs in gene	# sig SNPS p-val $< 0.1$	# indep sig SNPS $r^2 < 0.5$	p-value (10000 Permutations)
TP63	118	17	10	0.026
TP73	45	3	3	0.013

Breast Cancer - p73 SNP (rs6695978) MAF with age



Breast Cancer - MRE11A SNP (rs607974) MAF with age



<b>MRE11A (rs607974)</b>	<b>Women under 50</b>	<b>Women over 50</b>
# of T alleles in mothers	14	12
# of C alleles in mothers	30	60

p-value = 0.05

# ACKNOWLEDGEMENTS

## Evolution

Vladimir Belyi - IAS

Prashanth AK - IAS

Elke Markert - IAS

## Fertility

Wenwei Hu - CINJ

## Autism

Chang Chan - IAS

Asad Naqvi - IAS

D. Notterman - Penn State

## Stem Cells

Hideaki Mizuno - IAS

Geoff Wahl - Salk Institute

Ben Spike - Salk Institute