

KDRP 207 / PP PRES: BURNS – GENETICS

Genetics of Kawasaki Disease

Dr. Jane Burns describes her recent research into the genetic aspects of Kawasaki Disease.

28 March 2003

Cardinal Glennon Hospital, St. Louis

08:14:25

DR. IAN BALFOUR

Our first speaker is Dr. Jane Burns, who had the pleasure of hosting for Grand Rounds this week. She is currently chief of the division of Allergy, Immunology and Rheumatology at the Department of Pediatrics, University of California, San Diego. She is also the director of the Kawasaki Disease Research Institute. Dr. Burns' area of interest in Kawasaki Disease is in the genetics of the disease, and she will elaborate on that this morning.

Welcome Dr. Burns

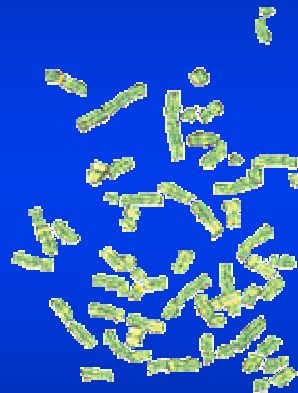
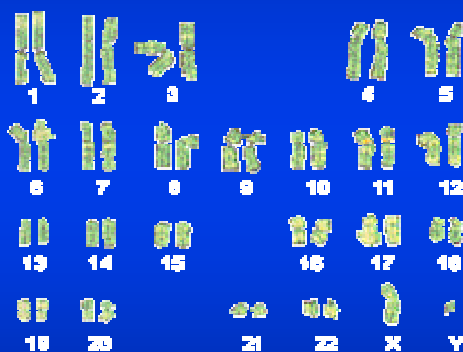
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DR. BURNS

Thank you, Ian and thank you to all of the other meeting organizers who have worked so hard to bring everybody together. Thank you also to **Donna Collins** for being an inspiration for getting together this group of people.

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The Genetics of Kawasaki disease



08:16:02

So we're going to talk about the [genetics](#) of Kawasaki disease and this is a controversial area, and I want to try to present for you, this morning a little bit of the evidence that makes us think that there may be a genetic component to Kawasaki disease.

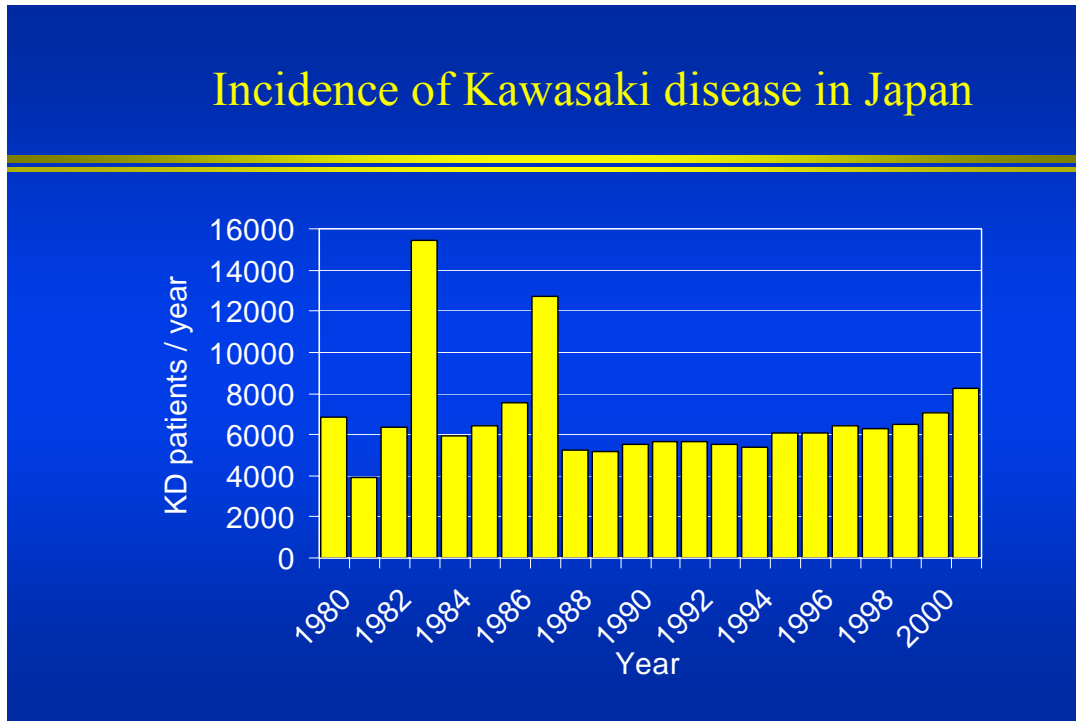
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Evidence that KD has a genetic component

- Increased incidence among Asian populations and among Asian/Americans

So we'll go forward with a list of some of the things that are commonly invoked as suggesting a genetic basis for this disease. And the first thing is that there is clearly an increased incidence of the disease among Asian populations and among [Asian Americans](#). And I don't just think this is just case ascertainment, but a true increased incidence.

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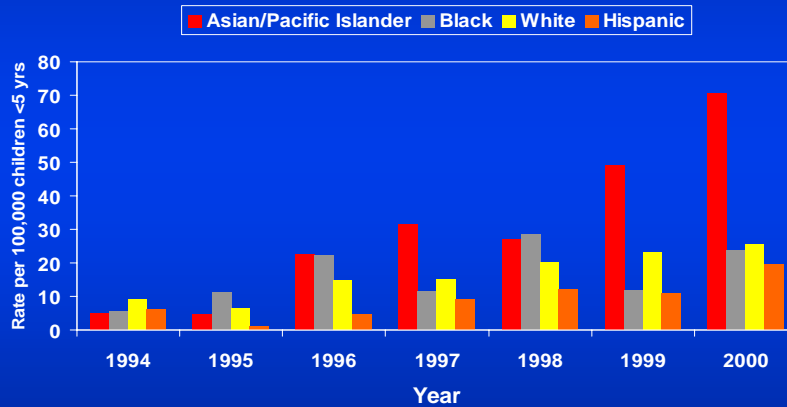


08:16:45

And if we look at the number of cases from Japan these are data from Professors [Nakamura](#) and Yamagawa in Japan. This is now a total number of cases in Japan. And I think we can easily see that the numbers of patients are slightly increasing here over 8000 patients in 2001, but clearly a very large number of patients, so this translates into an attack rate using various numbers but something around a 135 to 140 per hundred thousand children less than five years of age. So clearly a lot of cases.

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Kawasaki Disease incidence by race/ethnicity in children <5 years old, San Diego County, 1994-2000



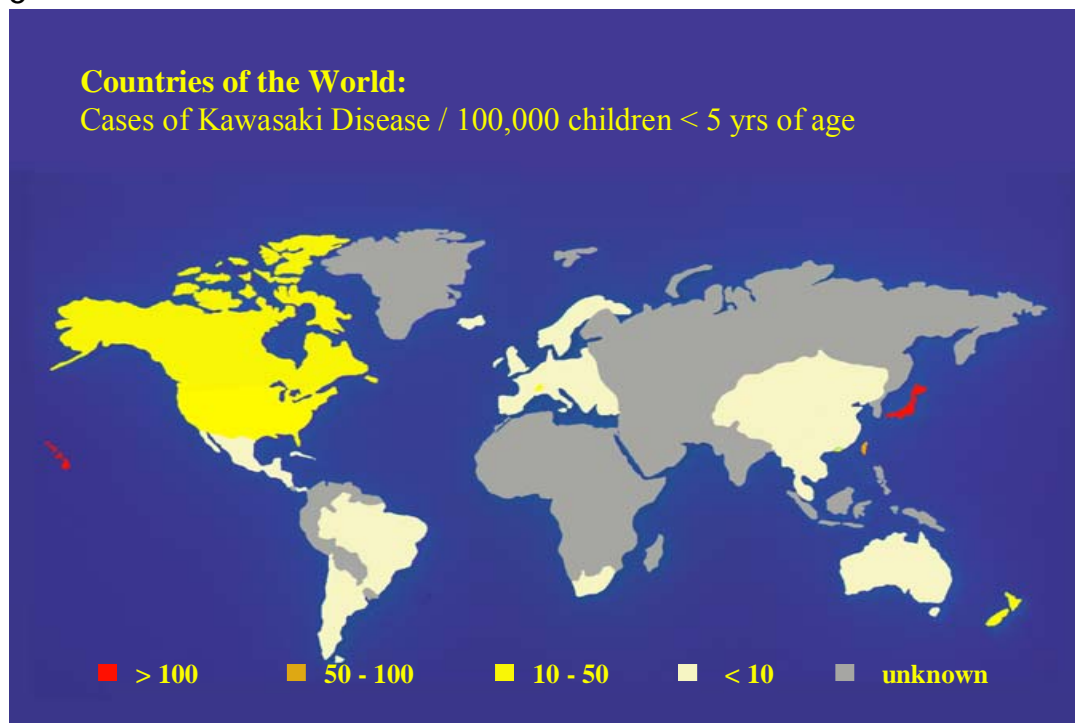
Note: These rates were based on the current estimates derived from SANDAG & Census 1990; Asian Rates include API/Other/Mixed

In [San Diego County](#) where we have the advantage of a monopoly in terms of pediatric care, in the geographic region that's delimited by San Diego County, it's very easy actually to track the patients who have been diagnosed and treated for Kawasaki disease. We also have the advantage of physical borders such as the Mojave Desert and the Pacific Ocean on either side of us. So we're a fairly contained geographic area.

08:17:58

And you can see that among our Asian-American Population and now, this is not just Japanese patients but also Filipino and Korean, which are probably our two largest Asian groups within the Pan-Asian Community. You can see that the attack rate for that group is somewhere up around 70,000. Now these are based on denominator data that were generated back in 1990 in the census but we've also looked at using more current numbers in the 2000 census. And we continue to see this very marked disparity between the attack rate in our Caucasian and Hispanic populations, we have a very small African-American community so we don't really have meaningful statistics there. But real disparity between our other ethnic groups and our Asian group.

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08:18:47

Now you have this slide drawn, because I wanted to think about the countries of the world in the distribution of the world of this disease. And unfortunately when we chose the map to use, we might have wanted to do it a little bit differently because we've got Japan over here in Red. Now there's a key across the bottom where the countries who have greater than 100 per 100,000 children, less than five years of age in attack rate, greater than 100, are shown in red.

08:19:18

And there are only two countries in the world, I'm not going to actually call Hawaii a country, I think I'll call it a State... but it's over here in the middle of the Pacific Ocean. And then the next highest level is 50 to 100 per 100,000 and you can see that they are very few areas. Only Taiwan actually and a few areas in China which you can't really see, on this projection, have very high attack rates. And then the yellow for North America, is 10-50 per 100,000.

Evidence that KD has a genetic component

- Increased incidence among Asian populations and among Asian/Americans
- Siblings of index case have 10-fold increased relative risk of KD

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08:20:00

And you can see that the distribution of countries that have a very high incidence rates is really confined to places that have very large Asian Populations.

08:20:13

So the other thing that's kind of in vogue to say that there is probably a genetic influence in who gets Kawasaki disease is the fact that [sibling cases](#) in an index case have a tenfold increase relative risk. Now this has been highlighted in several studies, all of them from Japan, first the occurrence rate in sibling simply counting a large series of patients taken from one of the nationwide surveys was found to be ten-fold increased.

Incidence of KD among siblings of an index case, Japan

- Occurrence rate in siblings of index case=1%; 10-fold increased rate among siblings
(*Yanagawa et al. Pediatrics 1998;102:e65*)
- 108 twins: MZ and DZ have almost same concordance rate (13-14%)
(*Harada et al. Am J Hum Genetics 1986;39:537*)
- 10-fold increased rate of KD among siblings; 50% were co-morbid with sibling case occurring within 10 d of index case; 2nd peak one year later; 30 twins, 13% concordant
(*Fujita et al. Pediatrics 1989;84:666*)

7

08:20:48

There have been very few studies actually of twins. Twinning is not very common for whatever reason in the Japanese population as compared to some other populations where twinning occurs at a higher incidence. And that's both for monozygotic and dizygotic twins. But the concordance rate is not something that a geneticist would jump up and down about and say that this was obviously indicating a genetic influence. So I think that the twin data has to be interpreted with a grain of salt, these numbers are very small, and so most geneticists would look at this and just write it off as saying too small to make a conclusion. And, and that may be in fact what we should do with this data, we really, really can't tell.

Evidence that KD has a genetic component

- Increased incidence among Asian populations and among Asian/Americans
- Siblings of index case have 10-fold increased relative risk of KD
- Emerging recognition of KD in successive generations

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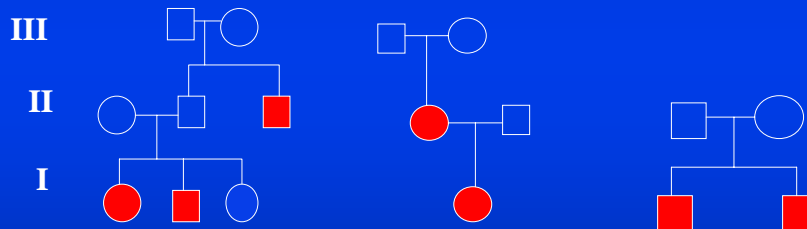
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And in a study that's frequently quoted by Fujita et al. There was also a tenfold increase rate of KD among siblings. What is unusual about that study is not something that we generally see in our community in San Diego, but there was about a 50% co-morbid rate where the sibling case actually occurred at the same time or very close to the same time as the index case. And again that's not something that we see in our community, I'd be interested to know, and other people are here from practicing in other regions of the United States.

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Examples of KD Pedigrees

Nakamura: 74 Japanese sib pairs
Burns: 11 American pedigrees



08:22:08

So, you know I think these data are not overwhelming, but we certainly do see sibling cases. And I think maybe what's convinced us more of the genetic link with Kawasaki disease is the emerging recognition of KD pedigrees. And I'll just show you some representative examples of those here. These are, are sort of randomly chosen to show the different kinds of KD pedigrees that we've collected, where we see two-generation cases here, in this would be an uncle, I believe. And here actually in the mother-child pair. And then the sibling pairs.

08:22:50

Now in our collection of these families we have 11 of them. We do try to recruit patients for a DNA study over the Internet. So we're trying to gather a larger number of these families, obviously they can be very informative later on for certain genetic approaches.

08:23:10

Dr. Onouchi and Nakamura initially working at the Genome Center in Japan at Tokyo University but now Dr. Onouchi has moved with his DNA samples to Riken <http://www.riken.go.jp/engn/> a private company, and is going to be analyzing 74 Japanese sib pairs. Using a positional cloning approach. So we await anxiously the answer from that study from Japan. It's going to take us a long time; I think in the United States to gather that many sib pairs.

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Single nucleotide polymorphisms (SNPS): The genetic basis of variability

- Genetic variation contributes to disease risk
- 30,000 estimated functional genes in humans
- Estimated SNP frequency:
 - 60-100,000 non-synonymous, non-conservative (amino acid-altering) coding region SNPs (cSNPs)

08:23:44

So we're obviously not talking about good-gene, bad gene. This is not Cystic Fibrosis; this is not sickle cell anemia. We are not looking at any kind of single or easy kind of genetic influence. We are certainly looking at a complex genetic influence. And we are undoubtedly looking at single nucleotide polymorphisms or other kinds of variation within the genome, that is in fact, what accounts for the differences among all of us sitting here in this room.

08:24:19

So just too sort of put in perspective the magnitude of the problem if you think you are looking for several small needles in a very large haystack you're right. Based on current information we think there are probably somewhere on the order of 30,000 functional genes in the human genome. The SNiP frequency that I've given you here; this is [Single Nucleotide Polymorphism](#), single base-pair substitution. These obviously can occur anywhere in the genome.

08:24:47

When they occur within the coding region of a gene. We call them "C-SNiPS" And these can be silent in that they can be somewhere in the [Codon](#) where they don't change. The amino-acid delegation for that triplet code. Or they can be amino acid altering. And there are probably between 60-100 thousand of these significant SNiPS that actually cause changes in the amino acid composition of the protein that result from the gene.

08:25:16

Of course there are lots of SNiPS that can occur in other regulatory parts of the gene that are non-coding sequences. And those may be equally important and we don't have good estimates about how many of those may actually exist.

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How do SNiPS exert their effect?

- Alteration of transcription factor binding sites
- Alteration of translation efficiency and mRNA stability
- Amino acid change that alters function, post-translational modification, or stability of protein
- Physically linked to important regions that influence disease susceptibility

08:25:30

So how is it that these SNiPS account for the variability that we see here in the room since we all share virtually 99.9% of our genome, why are we different? And these SNiPS have very profound effects. They are very small but they carry a big stick. And the kinds of changes that they can cause are first of all, alteration in transcription factor by binding sites. So this would be for SNiPS located in the five prime regions upstream of the coding sequence.

08:26:03

They can also cause an alteration of translational efficiency or affect mRNA stability, so a little more subtle, a little more difficult actually to test for.

08:26:14

They can, as I mentioned before, actually because amino-acid changes, if they occur within the coding region of the gene. And in that way alter function, they can alter post-translational modification, or impact the stability of the protein.

08:26:29

And finally the SNiPS that we identify could in fact be physically linked to other important regions in the genome that we haven't tested for, were unaware of or haven't explored. And it's really those other regions to where they are linked at that are important in terms of influencing disease susceptibility and disease outcome.

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Finding genes that underlie complex traits: The search for KD susceptibility loci

Approach #1: Positional cloning

- Step I: linkage and association
- Step II: Fine-mapping
- Step III: Sequence analysis
- Step IV: Functional tests of candidate genes

Difficulties: requires large number of sib pairs (>500), resource intensive

08:26:49

So when you try to approach the situation of going after the genetics of a complex set of traits, we'll call Kawasaki Disease. You have a couple of choices if you are going to do a candidate gene approach. The first choice is to do a case-control study design. And of the studies that have been published so far on Kawasaki disease have opted for this. It's very possible to do this and a reasonable approach in the Japanese population where most of the studies have been done. And that's because a lot of work by Professor Nakamura at the Genome Center in Japan has clearly documented that the Japanese are an amazingly homogeneous population.

08:27:32

So they've lived in isolation for centuries on their island. And in fact are genetically very very homogeneous. So the choice of a control group there is very straightforward. What you do is you look for differences in the frequency of a given alleles between affected individuals, those with Kawasaki disease, and the unaffected, unrelated controls.

08:27:57

The problem may be in Japan that if Kawasaki disease is very widespread, you may actually include people in your control group who have the disease and you are not aware of it.

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Association studies: Study design choices

1) Case-control study design:

- Detects difference in allele frequency between affected individuals and unrelated, unaffected controls

Problems:

- High false positive rate due to insufficiently stringent p value
- Controls and cases genetically different, "population stratification"

2) Transmission disequilibrium test (TDT):

- Detects preferential transmission of allele from heterozygous parents to affected offspring

08:28:09

You will see lots of these studies published, I'll review with you what the published literature is on those studies. And unfortunately the problem is that you need very large numbers. So most of the studies suffer from an insufficiently stringent P value, so we get this high false positive rate of associations, that may not actually stand up to the test of time and scrutiny with larger samples of DNA.

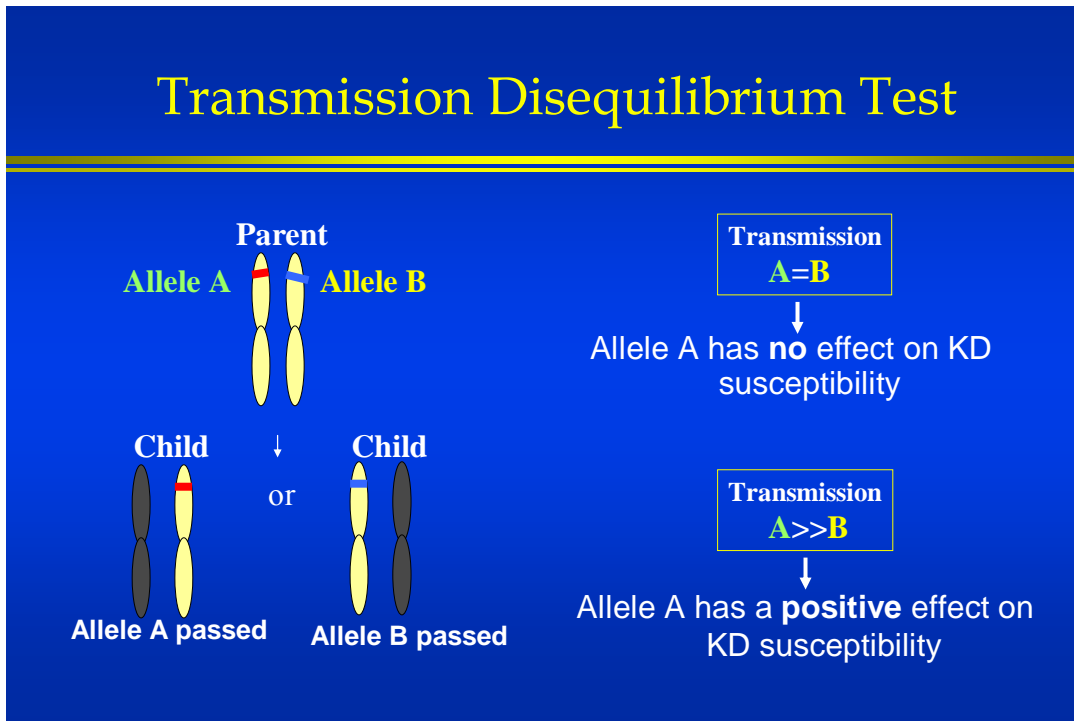
08:28:33

The other problem we have in this country is that the controls and the cases may be genetically different. So if I were going to take a case-controlled study design in an ethnically and genetically diverse population such as what we have in San Diego County, I would need separate control groups for, Filipino for Japanese, for Hispanic for Caucasian. It would be essentially undoable.

08:28:59

So we chose to take the approach using the [transmission dis-equilibrium test](#). I'm going to explain that in a little more detail but basically what this does is it

allows you to detect preferential transmission of alleles from heterozygous parents to the affected offspring. And the way this test works is diagrammed here.



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08:29:19

We show a heterozygous parent who can pass on either an allele A or allele B, to the affected child here. Now if allele A has no effect on susceptibility to Kawasaki Disease, then if you get a large enough sample you would expect those heterozygous parents who could pass either one of these alleles to pass them at an equal frequency. So the transmission then to the affected child of A and B would be equal, thus showing that Allele these alleles have no effect on KD susceptibility.

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Testing candidate alleles

- Determine frequency of allele in study and control populations or perform TDT analysis
- Replicate findings in independent study
- Test for biologic plausability
 - » Clone promoter region SNPs upstream of a reporter gene and examine effect on gene transcription

08:29:55

However if we see a preferential transmission, or skewing of transmission, and allele A is passed more frequently, then we can say that there appears to be an influence in Allele A, has a positive effect or a positive relationship to Kawasaki disease susceptibility.

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UCSD KD Research Program

- **Hypothesis:** Susceptibility to KD, development of coronary artery abnormalities, and response to therapy are influenced by genetic factors
- **Methods:** Multilocus genotyping of polymorphisms in genes that influence inflammation and cardiovascular health

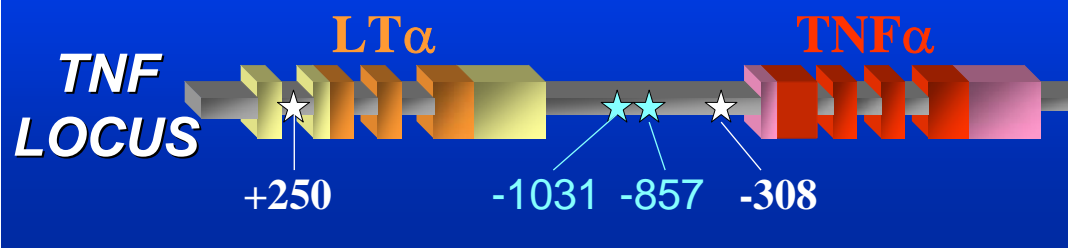
08:30:12

So at UCSD we've undertaken in collaboration, this is... it takes a village to do these studies. We've undertaken to adjust the hypothesis that susceptibility to KD, the development of coronary artery abnormalities, and perhaps response to IVIG therapy may all be influenced by genetic factors. And we are doing multi-focused genotyping so we've actually chosen candidate genes that we are pursuing. And we are using a transmission disequilibrium test format.

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Genetics of the immune response: TNF locus polymorphisms

- Multiple biallelic polymorphisms in TNF locus on chromosome 6
- Specific alleles at TNF α -308, -857, -1031 and LT α +250 associated with increased production of TNF α following inflammatory stimulus
- Only the TNF α -308 and LT α +250 polymorphisms studied in various diseases



08:30:45

Now in preparation for this study, we and also Dr. Kato and his group in Japan, did a preliminary study where we looked in a case-controlled format. So this is before the TDT Study. At polymorphisms that were influencing transcription of Tumor Necrosis Factor, in this case TNF Alpha.

08:31:10

We all know that TNF Alpha is a very important cytokine, co-inflammatory cytokine that influences a lot of the disease process in Kawasaki disease, and there are now many more of these SNIPS that have been identified in the upstream, (Vipine) upstream region, of the gene where they actually alter transcription factor binding sites, and so thus are candidates to potentially influence the amount of TNF Alpha native response to an [inflammatory trigger](#). There also happens to be another locus, upstream in an Exon up here for lymphotoxin alpha. And that also has an effect downstream on the transcriptional activity, in the TNF Alpha gene.

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Genotypic frequencies in children with KD compared with healthy control populations

Population	n	LT α +250			TNF α -308		
		A/A	A/G	G/G	A/A	A/G	G/G
Caucasian children with KD	46	0.59 ^a	0.37	0.04	0.00	0.17	0.83
Caucasian control population	105	0.36	0.51	0.13	0.04	0.26	0.71
Japanese children with KD	39	0.28	0.59	0.13	0.00	0.00	1.00
Japanese control population	39	0.44	0.41	0.15	0.00	0.08	0.92

^a Caucasian children with and without KD (LT α +250, A/A genotype: $p=0.013$; odds ratio 2.5, 95% confidence interval, 1.23 - 5.09). All other comparisons not significantly different. Statistical analysis using Fisher's exact test.

08:31:57

So these were candidates that were interesting to us and in a pilot case control study where we collaborated with Toyo Matsubara in Japan and included Japanese patients, as well as Caucasian patients, each with their own control group. We saw a skewing again this is, is in this kind of a study only looking for suggestive data, certainly not meant to be definitive with these numbers. But we saw a skewing of the occurrence of this A/A, for the rare allele the A/A genotype in Caucasian children with KD as opposed to the control population. We and also Kato's group in Japan saw no skewing or no differences within the Japanese population. But the Japanese, essentially the A allele is extremely rare in that population so you'd need very large numbers to be able to see an effect.

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Genotypic frequencies in children with KD and coronary artery abnormalities

Population	n	LT α +250			TNF α -308		
		A/A	A/G	G/G	A/A	A/G	G/G
Caucasians with CAA ^a	14	0.50	0.43	0.07	0.00	0.36 ^b	0.64
Caucasian without CAA	32	0.63	0.34	0.03	0.00	0.09	0.91
Japanese with CAA	11	0.27	0.55	0.18	0.00	0.00	1.00
Japanese without CAA	28	0.28	0.61	0.11	0.00	0.00	1.00

^a CAA=Coronary artery abnormalities.

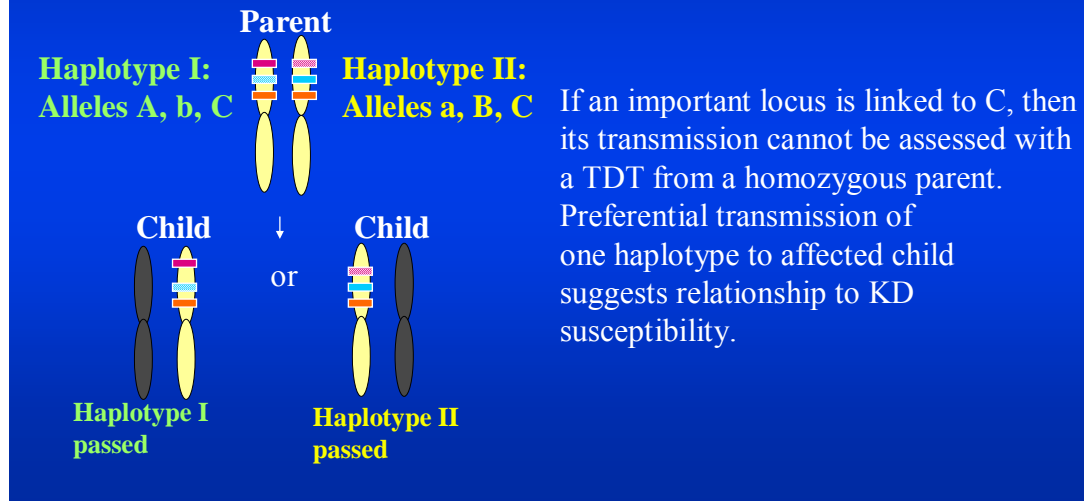
^b Caucasian children with and without CAA (TNF α -308 A/G genotype: p=0.044; odds ratio 5.4, 95% confidence interval, 1.07 - 27.01). All other comparisons not significantly different. Statistical analysis using Fisher's exact test.

08:32:55

And then when we stratified our analysis and looked just at children with coronary artery abnormalities, we saw an increased incidence of the A allele these heterozygotes in children who had coronary artery aneurysms. So this gave us a little glimmer of hope obviously not a definitive study but we thought that this was worth pursuing.

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Haplotyping: Inheritance of physically linked segments of DNA



08:33:21

And we then went on to design a bigger study. So I wanted to share with you first what has been published already.

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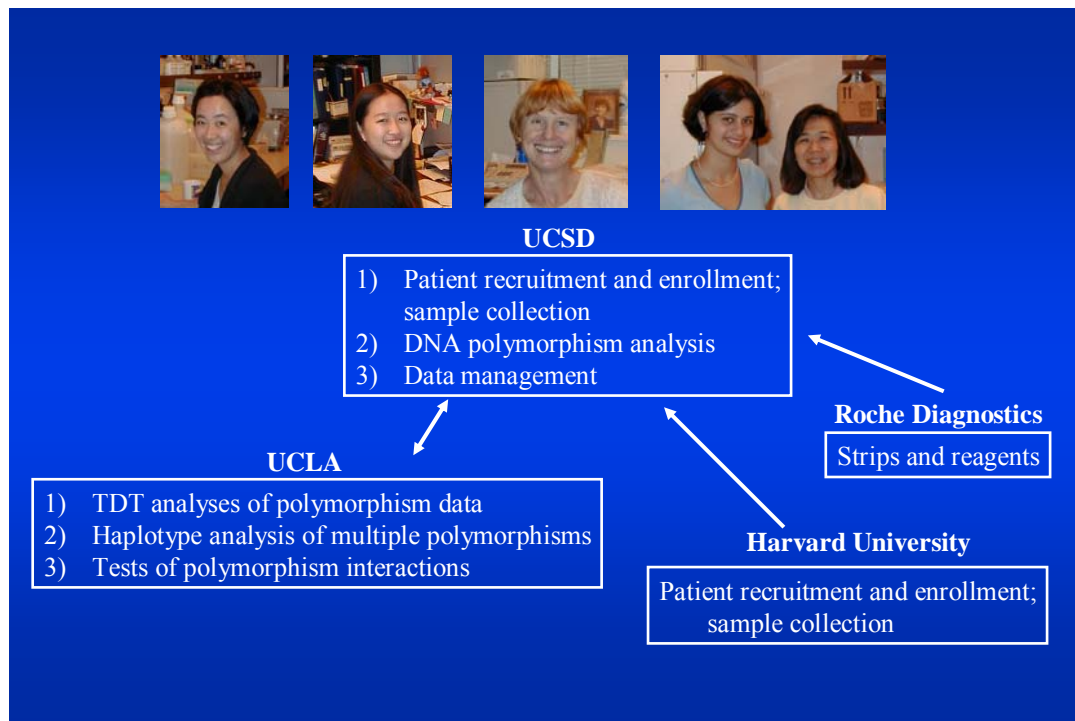
Gene	Polymorphism	Population, n	p value	Ref.
Angiotensin I converting enzyme	intron 16 insertion	Japanese KS, 16 CAA-, 20 CAA+	<0.01	Takeuchi, 1997
Tumor necrosis factor- α (TNF- α)	-1031, -863, -857, -308, -238	Japanese, 61 KS, 35 ctrls	NS	Kamizono, 1999
TNF- α	-308 A	Caucasian KS, 32 CAA-, 14 CAA+	0.044	Quasney, 2000
Lymphotoxin- α	+250 A	Caucasian, 46 KS, 105 ctrls	0.008	Quasney, 2000
MHC class I chain-related gene (MICA)	A4 microsatellite repeat in intron 5	Taiwanese, 70 KS, 154 ctrls	NS	Huang, 2000
		11 CAA+, 59 CAA-	0.005	
Methylenetetra-hydrofolate reductase	T 677	Japanese, 75 KS, 238 ctrls	NS	Tsukahara, 2000
SLC11A1	promoter GTn repeat, 134 bp	Japanese, 71 KS, 110 ctrls	0.0074	Ouchi, 2003

All of these studies I think from Asia. One from Taiwan and then the study that I just told you about in our Caucasian population. So the genes that have been looked at are the Angiotensin One converting enzyme polymorphism, TNF Alpha looked at by our group and Kato's group. MHL Class One, Methylenetetra-hydrofolate reductate, a gene that also goes by the name of Enramp One

08:34:04

All of these studies were very limited in numbers, used in case control format. But some suggestive or tantalizing data that may be worth following up in larger studies.

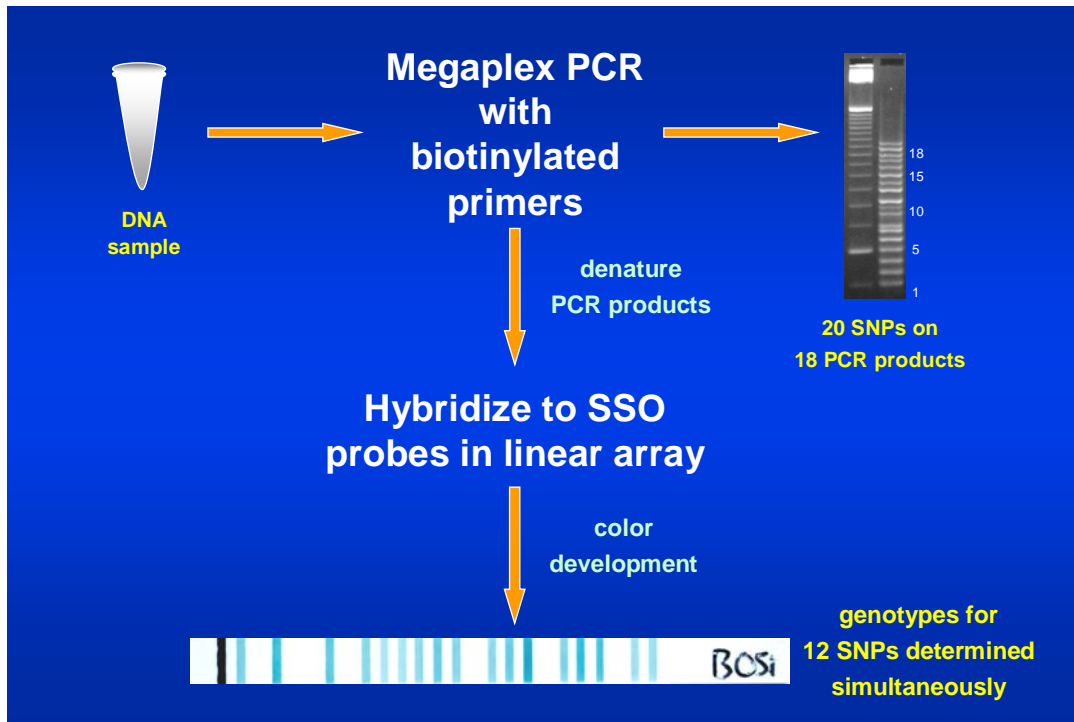
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08:34:17

So in our program, that is ongoing right now, we have patient enrollment going on at two centers, [Jane Newburger](#) and Rod Cindel at Harvard University are contributing DNA samples. We have the DNA Samples from UCSD, and we have a statistical center and our geneticists, Rita Kantor working with us at UCLA.

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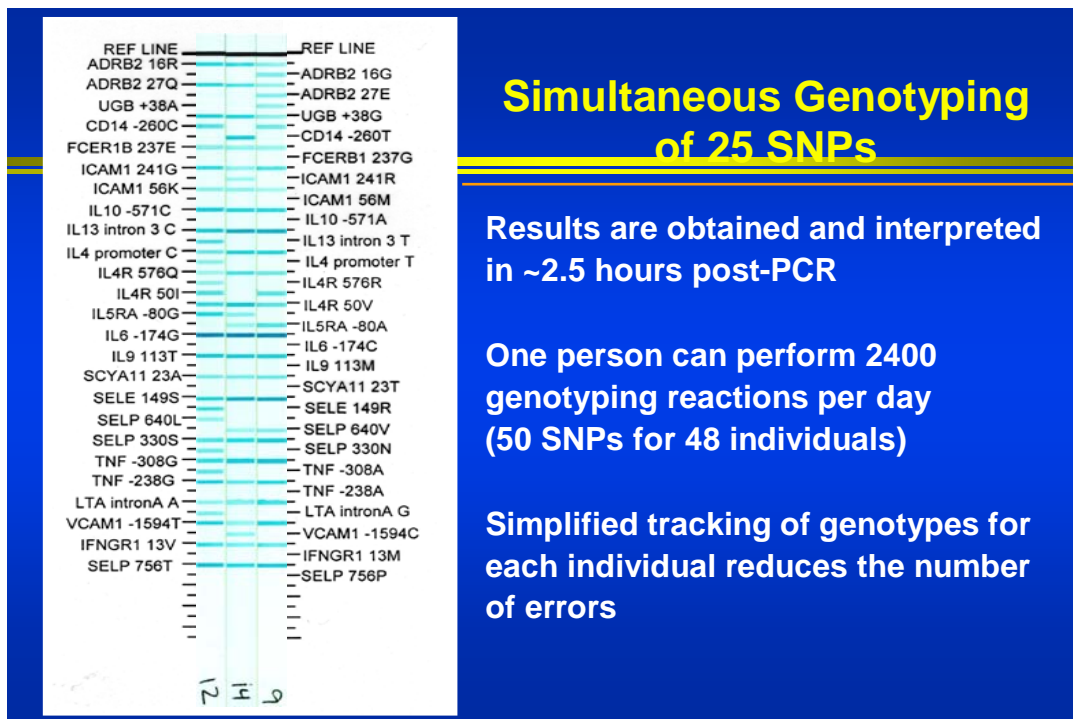
08:24:44

We are using a technology platform from [Roche Diagnostics Systems](#), and it works something like this. We take a 25-nanogram DNA sample. We subject it to PCR Amplification with up to 25, sometimes 30 primers that are [inaudible] in a single reaction. This whole process is has been worked out by Roche over several years. And they manufacture the strips as well as manufacturing the primer pools that we use for these reactions.

08:35:13

Once the amplification has gone one, this is what the TCR Products could look like but we just take those TCR Products directly from the tube. And we hybridize it using a color metric non-radioactive substrate. Hybridized it to a nylon membrane that has embedded in it the complimentary short sequence for that particular allele.

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So each one of those blue lines here that you see actually represents an allele, we put a template over it and we can actually read visually very easily the genotype of that individual. So this would represent, each strip represents the genotyping of a single individual.

08:35:54

I'm not going to go into any more details on the technique but I'd be happy to talk with anybody if you are interested during the break.

08:36:02

The screening that we're doing is for a hundred and seven different alleles. So that you can see that each of the studies that I showed you before was really looking at, at the most, one or two alleles. And now we are looking at 107. So a lot more comprehensive, given this technology base than what was possible before.

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Inflammation assay: 39 genes

- Cytokines
- Growth factors and receptors
- Chemokine ligands and receptors
- Adhesion molecules
- Migration
- Complement
- Nitric oxide synthases

And we have a number of candidate genes in the inflammation family and also candidates in genes that

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Cardiovascular assay: 36 genes

- Lipoprotein metabolism
- Blood pressure regulation
- Homocysteine metabolism
- Coagulation
- Cellular adhesion
- Inflammation
- Plaque stability

... have been linked to cardiovascular health and cardiovascular non-health.

27

Candidate alleles for susceptibility to KD

Preliminary analysis of 130 KD families

Gene	Location	Polymorphism (Allele 1, Allele 2)	Parents*	Allele 2 P:NP [#]	p-value
Chemokine receptor 5	3p21	Wt/deletion	30/254	8:22	0.016
Apolipoprotein (a)	6q26-q27	G121A	81/224	31:50	0.05
Complement component 5	9q32-q34	ile802val	121/258	72:49	0.05
Hepatic lipase	15q21-q23	C(-480)T	108/256	43:65	0.05
Glycoprotein IIIa	17q21.32	leu33pro	39/258	27:12	0.024

*Parents=heterozygous parents/total parents

[#]Allele 2 (uncommon allele) passed (P) or not passed (NP)

08:36:38

The results of our study to date are shown on this slide, which is a preliminary analysis of 130 KD families. And just to walk through with you maybe I'll take the example of a platelet glyco-protein 3A, this is an interesting allele that the adult cardiologists have been all over because of the relationships with coagulation problems, myocardial infarction, plaque rupture, and atherosclerosis is located on chromosome 17, and the way you read this table is you go over and you look at our parents, we had to genotype 258 individuals in order to find 39 heterozygous parents, we could only look at transmission dis-equilibrium when there is the option for the parent to pass either allele. So only the heterozygous parents are informative.

08:37:25

And you can see that there is a skewing of the transmission of the rare allele, which is the one that's been implicated in various issues in adult cardiovascular disease. Again, not in any way statistically significant given the multiple comparisons that we have to do for this study. But it's a beginning and we have to start somewhere.

08:37:45

So this study will be ongoing for the next two years, we'll be adding numbers; we're accruing about 100 patients annually. We are recruiting parents over the Internet, we have permission from the IRB at UCSD to be able to dialog with parents who contact us. So if you have patients in your practice and would like to refer them. To us for participation in this study or you think this is something that would be of interest to families. We are very interested in families with [aneurysms](#) or families with multiple affected members, they can contact me by email, I can't contact them. But if they contact me by email I can then initiate the process of sending out the DNA collection kit. We actually do it with mouthwash for older children and for the parents, younger children we have to get a blood sample. But we really appreciate your help. We can't do this project by ourselves.

08:38:38

One of the big advantages of the transmission dis--equilibrium test is we actually get information about Hapto Typing. And I think if we are going to have any positive results from this study, it's going to come from looking at Hapto Type inheritance. And what we are talking about there is because we have DNA on both parents, we can actually understand which alleles are physically linked on a given chromosome in certain settings and, and whether those linked alleles are being transmitted. So it's a whole different level of analysis that we can do, given our study design. And that's in process right, now, I don't have any of those preliminary data, I'm hoping to have those in time for the SPR meeting in May, where we'll be presenting this data again. But I wanted to give you a little update on them.

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Conclusion

- Genetic variation may underlie susceptibility to KD and outcome
- Different SNPs may be implicated in different populations
- Determining the role of genetic variability in KD will require international cooperation and collaboration

08:39:26

So in conclusion, I think it's very likely that [genetic variation](#) underlies both susceptibility to Kawasaki disease and outcome in terms of treatment failures. It may certainly be the case that different SNiPS will be implicated in different populations. So this study is going to have to be done in Japan and in the US. Which of course increases the work and increases the time and the money. And determining the role of genetic variability in KD is really going to require a huge scale international collaboration, which we're very pleased to have initiated with groups in Japan. But it's also going to require a lot of resources to get this done. So I'm going to stop there and I think questions will be afterwards during the break. Thank you very much.