DISCUSSION

Audience and Panel Participants: Harold Gordon, George Uhl, David Comings, Howard Moss, Ming Tsuang, Jag Khalsa, EricHollander, Ellen Witt, and EricDevor

Dr. Gordon: Thank you very much for the commentary and the ideas. We'll start a discussion on the genetic approach to biobehavioral etiology of drug abuse. I would like to give the first opportunity to those presenters especially involved in genetics research to comment, ask questions, and what-have-you and basically discuss the issues.

Dr. Comings: For a long time I've been intrigued with chaos theory as one explanation for why one can have similar input and get a wide variety of outputs on a particular set of variables. One could imagine, for example, that there's now a norepinephrine transport available and that it might be more related to anxiety disorders than it would be to drug abuse. So, if we find that there's a higher association between that transporter gene and panic attacks, then perhaps chaos is not as important as different genes going into the mix.

On the other hand, if we find again that there is no significant difference in the frequency in the transporter genes—assuming it's increased—of variance of the norepinephrine transporter in these different disorders (drug abuse and anxiety), then it would be another piece of evidence that the same set of genes can result in a wide spectrum of disorders. So, I think it's just going to take more work to sort these out.

Dr. Gordon: Any comments or questions now, especially from the folks at the table here who might be involved in this research who would like to raise issues?

Dr. King: I have a question. Are there any published data on preliminary results on the nature of association between the DRD2 allele variant in the control sample? Is it related to personality differences or to other aspects of behavioral impulsivity?

Dr. Comings: It's a good point. Dr. Jim McMurray, a member of our group, in fact looked at a group of controls in Loma Linda to stratify them according to the DRD2 polymorphism. He gave a questionnaire assessing defense style and found that those within the normal group

who carried haplotype I had more immature defenses than those that did not carry it.

Dr. Uhl: Do some of the people with more classic genetics background want to comment on the ways in which the genetics for a number of these disorders might, in fact, represent a unitary genetic predisposition, or to what extent fragmentary data exist in the literature that there might be genetic specificity (e.g., attention deficit disorder compared to substance abuse or Tourette's syndrome compared to ADHD)? I think the silence is probably because there haven't been many studies of these things in these groups of disorders yet.

Dr. Khalsa: You're showing the direction of the genes and the drug abuse in that direction. Can you comment on the other direction where drug abuse results in changes in genetic makeup?

Dr. Comings: I don't think it would result in changes of genetic makeup, per se, but it could certainly cause change to the neurophysiology. I would agree that, at least at a phenotypic level, there are some consequences of abusing drugs suggesting some arrows go in the other direction as well.

Dr. Uhl: But, I think it is important to stress that in some sense the idea of the genetic changes as a consequence of drug use is worth thinking about. The mutations that have made the RFLPs that are being studied were fairly specific because of the ability to trace them in races, actually, in the sort of population genetics sense. One can show that many of them predate the separation of—for example, African and European—races as they're currently recognized. So, these are not randomly occurring with high frequency series of point mutations; these are actually remarkably stable. But, because of the evidence for so-called founder effects in these populations these are tens of thousands, or more, years old.

Dr. Khalsa: That may be true, but I ask this because I have not come across any literature—really convincing literature—suggesting the genetic effects of cocaine or marijuana. If those mutagenic effects are there at all, they must be at very, very high dosages. As you well know, caffeine, for example, is known to be a chromosomal-breaking agent at extremely high dosages.

Dr. Raleigh: My question concerns gender differences. You've seemed to have identified a nice relationship between serotonergic and

dopami-nergic factors in terms of predisposing use and maybe accounting for severity. Are those data largely from males? And, if so, do you expect to generalize to females?

Dr. Comings: Well, it's interesting that you brought up the question of gender differences because that's an area of intense interest in our laboratory right now. We've actually used a technique called haplotyping, which is a different way of looking at yet another polymorphism of the D2 allele, and we find significant differences in haplotypes both by age and by gender.

You would expect that an autosomal trait would not have significant differences by gender. So, we think that this has an effect on some other aspects of human behavior, perhaps child rearing, who gets married and who doesn't, and so forth. But, depending on the disease we looked at, the controls tend to be equal in males and females. For Tourette's, of course, there were more males. The drug abusers in the addiction ward were all males. But, yes, that's why I put sex in there. I think sex and age are going to play a very important role in these things.

Dr. Moss: I'm somewhat cautious when I see these tables with 15 and 20 different studies displayed as indicative of an effect in substance use disorders. There's a wide variety of approaches that have been utilized to characterize the same phenotype of interest. For example, on some of Dr.Comings' slides he noted drug abuse as alcoholism. On other slides, substance dependence disorders would be contrasted with substance use disorders. Right now we're in another state of flux in that DSM-IV is being implemented and that really establishes a whole new set of criteria, diagnostic clinical criteria, for psychoactive substance use disorders. How are we going to handle this degree of heterogeneity in the pheno-types that are of interest to us for genetic etiology?

Dr. Comings: This problem is one of the reasons, in our Tourette's syndrome study, that we didn't use the DSM-III diagnosis. We used, in fact, the variables that go into the DSM-III diagnoses independent of criteria because the criteria keep changing on us all the time. Every one of those variables was highly significant; so, I think that transcends the temporal changes in the criteria somewhat.

Dr. Moss: Physical dependence was as salient as the psychosocial dysfunction domains?

Dr. Comings: Well, to partly answer your previous question, in the study we've most recently done, we've used classic DSM-III-R criteria for alcohol abuse, alcohol dependence, drug abuse, drug dependence, and the combination of the two. So, in that study we did use the DSM-III-R criteria. In the Tourette's syndrome study, I simply took the DIS questions and looked at each question individually. The answer to every one of those questions was significantly greater with greater genetic loading for the Tourette's syndrome gene.

Dr. Moss: Let me sort of give an example of the way this can be problematic. The way DSM-III-R is structured, the psychological dependence and physical dependence are subsumed under the rubric of the dependence disorder. Abuse conditions, for example alcohol abuse where somebody who drinks alcohol on weekends and repeatedly drives their car to get home from the bar, would get a DSM-III-R diagnosis for alcohol abuse. It's a distinctly lower severity kind of syndrome than the dependence categorization. I'm not sure that it's completely legitimate to lump together individuals who have that residual abuse diagnosis, which may be a much milder variant of the condition, with the people who have the full-blown syndrome of dysphoria that we consider dependence.

Dr. Comings: I agree with you completely. I know the data were presented quickly, but we differentiated between alcohol abuse and alcohol dependence. The alcohol abuse category, if anything, showed a lower D2A1 prevalence than the general population, and alcohol dependence jumped up 12 percentage points. So, I think you're right, these are relative.

We've also looked at, with either just a quantity frequency—how much substance has been consumed over what period of time—or with quasi-DSM criteria, and they both give the same answer in terms of the gene association, which is a modest but noisy effect. It's certainly likely that if one had the right behavioral questions to address then one could get a stronger effect. We hope to be able to, in some sense, perhaps parse out the effect of specific genes that can help make the DSM revisions more specific. They're not, as far as I know, informed with a whole lot of genetic information; they're fairly set up to make clinical diagnoses. And perhaps also, as more specific genetic influences become available, that will allow the environmental features to be put into sharper focus individually and distinctly.

Dr. Hollander: Dr. Comings, you draw an association between Tourette's syndrome and substance abuse and a very wide range of

impulsive and compulsive spectrum disorders. It may be that you may be able to look at certain kinds of subgroups within the impulsive and compulsive spectrum and find that they may not all be associated with the same risk of substance abuse. For example, pathological gamblers, or Tourette's syndrome, or certain other disorders that have high impulsive and aggressive features may differ somewhat from, let's say, certain subgroups of OCD patients or, for example, anorexics who may have a lower likelihood than the general population of engaging in substance abuse. So, you might want to look at certain kinds of subgroups within that whole impulsive/compulsive spectrum.

Dr. Comings: As a matter of fact, we did that, and I didn't present those data here. But, just to give an example, we've been impressed for many years by the compulsive eating in many of our Tourette's syndrome families. But, when we used compulsive eating as a variable relating to the diagnosis of Tourette's syndrome and Tourette's syndrome nonprobands, it was barely significant.

But, when we looked at it as a subclassification of those who had OCD of the Tourette's syndrome, it was overwhelmingly significant—10-9. So, if you looked at the whole group, it was barely significant, and, if you looked at a subgroup, it was highly significant. So, we call this "association by association." It's clear that the Tourette's syndrome gene plays a role in OCD, and we looked at just that group of Tourette's syndrome patients. Their frequency of compulsive eating disorders was off the scale. So, I agree with you. Yes.

Dr. Gordon: Dr. Uhl, you brought up the potential problem with the gene variants in several populations, several studies, around the world. I noticed France was low, or somebody else was high. This might be a problem, but what is the implication? How does one get around it, and can we still learn anything about substance abuse and the relationship to these genes? I just picked that particular difference, but there are others. What's the implication?

Dr. Uhl: In terms of study design, I think it has very important implications. Clearly, the controls are as important as the probands, the individuals that are accessed, and that's, you know, substantially more true for association than just about any other study. If one had an ideal strategy and could go into a population-based sample and identify both a substance abuser subset and a control subset in a population-based fashion, that would be ideal. The second best is to try to make sure that the control group studied is as closely representative—in terms of all the demographic features that you

could imagine—of the, for example, substance abusers, as possible. Within caucasian ethnicity, variation exists. It's tempting to combine controls from all over the world, but that may not, in fact, produce a mix that's representative of your local Tourette's, substance abuse, attention deficit—whatever population. Control is key.

The history of the allelic association studies is hugely flawed based on inadequate control comparison groups and a number, even in alcoholism, with A/B/O blood group comparisons, and so on. A number of asso-ciations thought in the past to exist have subsequently been invalidated. So, I think this is one of the things that raises cautions about these results, as well as the increasing optimism. Because of replication in a number of different centers some of this association may, in fact, be real.

Dr. Comings: I'd like to comment on that, too. In our studies in California we generally find somewhere between 8-12 different national groups among the four grandparents, so it's really quite a heterogeneous mix. I think when we do these studies in the United States, we hope that some countries like Germany, France, England, and Japan will then pick them up and look at them within not only racial groups but within single ethnic groups, and that has been done. In fact, the most recent study—it just came out of France—was the most positive of almost all the studies that I've seen. The A1 allele was, I think, 22 percent in controls and 43percent in alcoholics, which is overwhelming. And even in Japan, where the frequency of the allele is very high—around 60 percent in controls—they found a highly significant association with severity.

The other issue about controls is that the Gelernter group used relatives of patients with Tourette's syndrome in their control group. Now, when we studied relatives of patients with Tourette's syndrome, the prevalence of the D2A1 allele runs around 40 percent. You have to be careful what control group you take. Jim McMurray and I have been interested in controls. He looked at a group of physicians and PhDs in Loma Linda compared to non-physicians and non-PhDs, and there was a significant difference in these alleles between these two groups. In fact, when he did the psychological tests, he found that the physicians and the PhDs tended to have a history of some fairly aggressive behaviors in childhood which then, as they grew up, channeled it into competitiveness. Maybe that's why they're doing what they're doing. So, even among the controls you have to be careful within one racial group.

Dr. Tsuang: I would like to rephrase the question that Dr. Uhl emphasized in terms of specificity. And I want to ask Dr. Comings about his current impression, and also in response to DSM-III and DSM-IV, how to characterize phenotype? I think, here, commonsense of doing clinical genetics will tell us traditionally that the manifestations of the different symptoms and signs are actually phenotypic heterogeneity. You mentioned about the common or predisposition leading to various mani-festations of symptoms or signs. This may be one way of looking at it.

The other way is to look at the genetic heterogeneity. If there has not been molecular genetic research in this area, I'll bet all this is phenotypic hetero-geneity. Look at the case with Alzheimer's disease. Before the discovery of chromosomes 21, 14, and 19, no one would stick out their necks to say that is the single gene. They were always talking about the polygenic and the genome manifestation—and in the case of Alzheimer's may manifest these clinical symptoms, may manifest obsessive-compulsive symptoms, may manifest all kinds of symptoms—and that is what we call phenotypic heterogeneity.

Yet, recent advances in molecular genetics have led me to think that even though it looks like phenotypic heterogeneity, it may be part of that syndrome—may be due to genetic heterogeneity. So, how to compromise between DSM-IV and whatever the Chinese cooking style of the diagnoses is that we shouldn't forget that the phenotypic manifestations are so variable and so unstable, and from the longitudinal followup of what we have done it's changing. Schizophrenia, the same thing, from paranoid schizophrenia you cannot rely on them. So, one factor that should be included is what geneticists call endophenotype, which essentially is not observable but with the biological or neurochemical with imaging and neuroanatomical studies. You may be able to get a grasp on what are the phenotypes; what is the specific phenotype we use. So that this spectrum concept of the genotypes manifesting typical cases of drug abuse or dependence, then within the family you have to look into what are the subforms of the aggregate of the dependence, even not meeting DSM-III criteria. So, family data are very important. Biological data are important. Then, if we can identify specific genetic predisposition in subforms of those, we may be able to identify those carriers, gene carriers, who may not have any symptoms at all. This is a very complex issue.

So, I'd like to ask both of you, what's your bet? Are we really looking at the phenotypic heterogeneity, or genetic heterogeneity, of drug abuse?

Dr. Uhl: Dr. Comings mentioned something I think that is worth stressing again: The reason why a linkage study might be negative, even when an association strategy would work, actually relates to this concept of genetic heterogeneity. The linkage method is a lot more susceptible to genetic heterogeneity than association methods. If one starts with a few families and if there are heterogeneous genetics in the different families, then that's going to reduce the power of a linkage approach much more than genetic heterogeneity reduces the power of an association strategy. I'm not sure that there's no reason—and I would argue that there's some fragmentary evidence to suggest both—why there couldn't be both genetic hetero-geneity and phenotypic heterogeneity. I think that's the likely scenario, in fact, unfortunately to account for the clinical phenomena of substance abusers.

Dr. Comings: There's several issues you brought up. One was the phenotypic heterogeneity. We think, I think, of Tourette's syndrome as a tridimensional spectrum disorder. Patients themselves have a wide range of phenotypes; their family members have a wide range of phenotypes. Over time, the natural history of Tourette's syndrome is not to start with tics but to start with attention deficit disorder. Then, a couple of years later, they start developing tics. Those tend to go away in adolescence, and then they have trouble with alcohol and drug abuse and conduct disorder. Later, in their 20s, they're having problems with panic attacks and anxiety. Later in life, they're having trouble with chronic depression. So, depending on what age you look at, they can have completely different phenotypic expression. Yet, the genetic underpinnings are fairly similar regardless of what age you get them or look at them.

The other issue about linkage studies, the reason I think they are failing, is just this issue of genetic heterogeneity. Linkage studies can pick up a gene that is genetically heterogeneous, but it's predicated on the assumption that there's going to be only 2 to 3 genes involved. However, in fact, 5 to 20genes may be involved. As a result, the power of linkage study is just drastically reduced. But this does not happen with association studies. All you need to do is look at a large number of probands and stratify them properly by race. I think you can pick up a 1 to 10 percent effect with association studies that you could never pick up with linkage studies.

Dr. Witt: I'd like to know what is the state of the statistics geneticists use to deal with genetic heterogeneity?

Dr. Uhl: My impression is that people have done models looking at various assumed genetic heterogeneities and looked at—for example—reductions in power in the work of Dr. Gershon—reductions in power and association linkage studies and so on. Is that your question?

Dr. Witt: Are there models to account for multiple genetic disorders looking at cause rather than the correlation among multiple genes? Or is that just something that needs to be developed?

Dr. Uhl: The quantitation?

Dr. Witt: Yes. The quantitation.

Dr. Uhl: You can make these assumptions, I guess. And you can look at the effect on different genetic parameters. But that doesn't seem to address your question.

Dr. Comings: One of the approaches is to use a thing called sib-pair analysis or the haplotype relative risk approach. These require parent/child sets. The idea with the haplotype relative risk technique is to take parents with an affected child and put the two genes that that child has inherited in one group and the other two genes in another group that serves as your control group, then determine if they are significantly different.

The beauty of that approach is that it's totally independent of racial and ethnic differences, so you would think, "Gee, this is an ideal way to look at these issues." But the drawback of that is that, where you had 100cases with the association study, you now need 300 samples for the haplotype relative risk procedure. You have to get all their parents together, which can be very difficult in older people. Finally, about half of those cases are going to give you no information because both parents have the same allelic makeup. So, now you need 600 samples to get the same thing that you could get with 100 probands in the association study. These issues of heterogeneity and how to get at them with linkage studies have been intensively investigated, but they all have problems.

Dr. Devor: I just wanted to make a couple of comments—methodologic comments—bearing on what Drs. Comings and Uhl

have said. The implicit recognition that I hope comes out of this, that dealing with a complex heterogeneous developmental phenomenon is a mistake that went over the heads of a lot of people working in alcoholism. There was the implicit recognition that there was a complex developmental phenomenon going on, and then everybody jumped on linkage studies, which are inappropriate to that kind of disorder.

The model that I hope can be used as an overall heuristic model is one in which things like incomplete penetrance—which is also just as deadly to a linkage study as is genetic and etiologic heterogeneity—can be accounted for by the fact that there may be genes (for example in a dopaminergic system) that give you an underlying risk to illness through a complex of illnesses and through the environmental and subsequent genetic gene-gene/gene-environment interactions that channel into the phenotypes that we see at the endpoint. But, the phenotypes at the endpoint do not necessarily have to be one-for-one specificity with a particular allelic variant or particular quantitative trait.

One case in point, and one that I've been fairly close to for awhile, is the situation with MAO-B. The criticism of MAO-B is that it seems to be lowered in everything. However, when you start properly stratifying within either a family or an association study for severity and concomitant psychiatric illness, what you find is that you have overlapping decreasing quantitative distributions of MAO-B activity that correspond to increasing levels of severity of the illness—say, for example, alcoholism—and increasing levels of secondary psychopathology in the unaffected family members. It's this that is leading me to believe that there are these under-lying genotypes—the primary genotypes—that lead to a general risk, giving a context on which other genes and environmental effects then take hold and channel the individual—it may be an improper way to put that—but channel it into what we now look at as the end phenotype. Comment if you will.

Dr. Comings: That's exactly the same effect we were seeing with the dopamine genes. Thank you.

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