So I’m going to be talking about genetic risk factors and biomarkers for concussion. My disclosure is that I receive financial compensation from the NFL Benefits Association and this was to develop a neurocognitive benefit program for retired football players who were suffering from cognitive impairment.

The learning objective is identify CSF and blood biomarkers which may assist in the diagnosis and assessment of sports related concussion. And I think it’s important to emphasize assist in the diagnosis because as was emphasized earlier today the diagnosis of concussion is a clinical diagnosis, there is no secret or magic potion or some magic bullet that helps you make the diagnosis. There is things that can assist in making the diagnosis but it is a clinical diagnosis. And I’m also going to talk about those genes that may influence the predisposition to concussion as well as those genes that may influence recovery from acute and chronic traumatic brain injury.

So there is a variety of different types of biomarkers, you can have blood biomarkers, CSF biomarkers, genetic biomarkers and neuroimaging biomarkers. I will not be talking about neuroimaging biomarkers, that’s a whole other discussion. In terms of blood biomarkers, a blood biomarker would be idea because it’s easy to draw blood, I think CSF biomarkers are much more problematic in the sense that if you have an athlete that you are evaluating for a concussion I don’t think this is a good idea to do a spinal tap. If you imagine what kind of headache they’ll have after that.
So what would be the idea biomarker be? Well it needs to be sensitive, it needs to be sensitive to traumatic brain injury, and it needs to be specific. So you don’t want to have a biomarker that will become positive with any type of brain insult, you want it to really be specific to traumatic brain injury. It should reflect disease activity, so this biomarker goes up when the disease is more severe and when you treat the patient or when the patient recovers this biomarker should improve.

Now there is one disease that I want to highlight where biomarkers have played a very important role, although it’s not directly related to acute traumatic brain injury it may have similarities to chronic traumatic brain injury and that is Alzheimer’s disease. And in Alzheimer’s disease there is three stages, there is a preclinical stage where the patient is asymptomatic, then there is a mild cognitive impairment stage and then there is the actual stage where there is full blown dementia. The MCI is also known as a prodromal phase. And with the biomarkers some of the biomarkers in Alzheimer’s disease are positive before you actually develop the disease. And so we can get using amyloid PET scan we can actually identify amyloid in the brain of someone that may be going on to develop Alzheimer’s disease. So that’s what we need in brain injury and in particular chronic traumatic encephalopathy, some type of biomarker that will reflect disease activity so that we can diagnose it in life.

So in terms of biomarkers there is a variety of biomarkers and they typically reflect the part of the nervous system that’s either injured or involved in the process. You can have glial fibrillary acidic protein, S100 beta associated with injury to the astroglial cell, you can have tau neurofilament light protein, myelin basic protein and microtubular associated protein that may be
associated with damage to the axon. You could also have ubiquitin-C terminal hydrolase L1, and spectrum breakdown products and neurons specific enolase reflecting injury to the neuron or the cell body. Then there is other ones that are available, amyloid precursor protein and beta amyloid protein may reflect injury or involvement of the distal axons or axon terminals where you could actually get amyloid plaques. As was alluded to in the previous talks in acute brain injury sometimes you get amyloid deposition and APP can be a marker of axonal injury. And then there is other possible biomarkers, CPK-BB, this is creatine phosphokinase, there is 3 isoenzymes of this, there is one for the brain, one for muscle and one for the heart. And actually the CPK-MB is for – is the isoenzyme that they use to document myocardial injury. So this may reflect injury to the brain. And then there is a neurotrophic factor, brain derived neurotrophic factor that some people have looked at which is a protein that supports the survival of neurons in the nervous system.

Okay, so the most extensively studied blood biomarker has been S100 beta, however the results have been variable. One of the issues with S100 beta is that it needs to be collected within 4 hour of the injury, and the other problem with S100 beta is that there may be extracranial sources of this biomarker.

This is a study that looked at the marathon runners and 18 marathon runners without evidence of brain injury and they found an increase in the post-race serum S100 beta and they thought this was from extracranial sources. They also looked at GFAP and they didn’t find any changes. So I think
this might be a problem when you interpret data looking at S100 beta because there may be extracranial sources. And as I mentioned before, you have an ideal biomarker it needs to be specific.

Blood biomarkers in boxing, this is an earlier study that was done in 1992 where they looked at CK-BB isoenzyme and they compared it in boxers and cyclists and found that the BB had increased significantly in boxers compared to the cyclists. And blows to the head in a proportion of these individuals correlated with the rise in BB, CK-BB. And they postulated that this elevation in CK-BB may be related to breakdown of the blood brain barrier. As far as I know no one has really followed up on this study, I’m not certain why. One possibility is the fact that this indicates that there is a breakdown of the blood brain barrier but that may not necessarily mean that that person has brain damage or has cognitive impairment or has sustained a concussion.

Another study looking at boxing, this group, Zetterberg, they looked at a panel of serum biomarkers 2 months after nonparticipation in boxing and they looked at 44 amateur boxers compared to controls and they found elevated NSE in that group, that’s neuron specific enolase, and they found no differences in brain derived neurotrophic factor and S100 beta.

A more recent study and I looked at this paper with great interest because I think it might show some promise but I think it needs to be duplicated, this is a recent study that was published looking at 30 Olympic boxers. And blood was drawn on two occasions, either 1 to 6 days after a bout and then at least 14 days after a period of rest. And the boxers compared to the controls did have an elevated plasma tau level compared to the controls, and then when they rested the tau level actually went
down. They didn’t find any elevations in the S100 beta, beta amyloid, BDNF or GFAP. And they concluded that repetitive injury may result in neuronal injury. The only concerning part about this study was that it didn’t correlate with CSF biomarkers. I’m going to talk about this study, a previous study related to the same cohort where they looked at the CSF, and somehow this plasma does not correlate with the CSF so it makes you wonder how reliable it may be.

This is a study looking at soccer, and again they looked at S100 beta, NSE among female soccer players before and after the game. And both of these biomarkers were elevated after the game, and there was a correlation between changes in S100 beta and actually hitting the ball.

A similar study has also been done in ice hockey and in basketball where they looked at S100 beta and NSE among hockey players and basketball players and there were significant changes between the pre and post-game serum 100 beta levels. And there was no changes in NSE.

This is another study looking at boxers, and they looked at 4 biomarkers in amateur boxers and the analysis was done 7 to 10 days after a bout and then 3 months later after a bout, presumably when they weren’t boxing and they also assessed the number and severity of the blows by energy when they had their boxing competition. And in this study it was noted that the NFL, total tau and GFAP all increased after the boxing match and that NFL actually remained elevated after 3 months of rest and they concluded that amateur boxing was associated with neuronal and astroglial injury.
And more recently in this cohort of 30 amateur boxers that I mentioned earlier where they looked at the plasma tau this is a CSF study and in this study the CSF was collected again 1 to 6 days after the bout and then 14 days after a rest period, and then the only thing they found here was that the CSF NFL are correlated with boxing exposure. That doesn’t necessarily mean it correlated with function or neurological function but it did correlate with boxing exposure. And none of these biomarkers in this study correlated with what they found in the plasma in the previous study that I mentioned. They also looked at the beta amyloid and tau in this study and they didn’t find any difference between the control group and the boxers; however they did note that beta amyloid was more variable in the boxing group.

Now people have also looked at soccer players, this was a study where they looked at soccer players and these players actually performed headings of the ball and they didn’t find any difference in the CSF S100 beta between those that head the ball and the controls. I wouldn’t really expect to find a biomarker to be positive associated with heading the ball simply because the amount of trauma is probably minimal and to have something sensitive enough to pickup changes in the serum or CSF after heading the ball I think would be very difficult.

Now let’s talk about genes influencing non-sports related traumatic brain injury. There have been various number of genes that have been looked at that may influence outcome after traumatic brain injury and this is not in sports, this is in just in the general traumatic brain injury literature. Some of the genes have included APOE promoter gene, COMT which is catechol-o-methotransferase,
dopamine D2 receptor. There is other ones such as interleukin, P53, angiotensin converting enzyme and CACNA1A.

Now it’s important to emphasize that when you are looking at genetics and you want to look at how it may influence the outcome following brain injury it’s important that you are evaluating what you think that gene may be involved in. So for example if you were going to look at BDNF gene you would probably need to assess plasticity and regeneration, you might want to assess processing speed or working memory. If you are looking at COMT which influence the amount of dopamine in the brain you want to look at functions that are related to dopamine, which might be executive function or working memory or attention. And if you are looking at APOE again you want to look at recovery and look at regeneration. So for the most part I might not expect a gene like APOE to be influencing the risk of concussion but I might expect it to influence the recovery from concussion.

So the most extensively studied gene has APOEe4. Now APOE has 3 isoforms, e2, e3 and e4. Each individual gets one copy from each parent, e4 is the gene that’s been associated with increased risk of Alzheimer’s disease and in the Alzheimer’s population the carrier rate for the e4 allele is probably upwards of 40%. In the normal population it’s about 25%. Well this gene has been associated with unfavorable outcome following general traumatic brain injury which has included outcome following coma, the duration of posttraumatic unawareness, on CT scans of more moderate and severe brain injuries e4 was associated with larger documented hematomas. It may increase your risk of posttraumatic seizures. It may also increase the frequency of contusions. In other cases they’ve noted that individuals that have had traumatic brain injury if they had the e4 allele they also
had lower cognitive function and it may also increase your risk of dementia, a subclinical dementia following traumatic brain injury.

In a metaanalysis of 14 cohort studies the e4 was not necessarily associated with injury severity but it was associated with poor outcome at 6 months. So I think that the important thing to realize is that the e4 allele may be influencing recovery as opposed to the initial severity of the injury. And that makes sense because one of the functions of APOE is to carry cholesterol in the brain which is used in the healing process. So let’s review some of the studies that have looked at genetics and sports concussion whether it be acute or perhaps the more punitive chronic affects.

One of the first studies looking at acute concussion was Terrell and colleagues, they looked at 195 college athletes, football and soccer players. And there was a cross-sectional study and they looked at the association between APOE, APOE promoter and tau. And then they had the athletes provide a self-reported history of whether they had a concussion or not. They found that one of the polymorphisms in the APOE promoter was associated with a 3-fold increased risk of having, or self-reporting a previous concussion and a 4-fold increased risk of self-reported history of concussion with loss of consciousness. They didn’t find an association with APOE or tau. I think one of the limitations of the study is that it was self-reported and you know how inaccurate that could potentially be. The other thing is that function wasn’t assessed either during this, so you don’t know really how severe the concussions were.
Another study, this is a prospective study of 318 college athletes and they compared concussion rates in athletes with and without the e4 allele and they didn’t find any association with the e4 allele. Again as I mentioned, with e4 I wouldn’t necessarily expect it to increase your risk of having a concussion, I would suspect that if it was going to be involved it might affect your recovery rate, so I think it’s really important that when you design these studies to look at genetics that you are really assessing what that gene is related to. So if you were going to do a study of concussion in athletes and you wanted to see the relation with APOEe4 I think it would be worthwhile to look at recovery.

This is another study looking at APOE and this is Tierney and colleagues. Actually this is a multicenter cross-sectional study evaluating association between carrying one or more of what they call rare alleles. A rare allele would be the e2 or e4 allele, it would be one of the polymorphisms of the APOE and one of the polymorphisms of tau. And they found that the athletes carrying 3 or more of these rare alleles are 9.8 times more likely to report a previous concussion and concluded that carriers may be at a greater risk of concussion. And athletes carrying the APOE promoter gene were 8.4 times more likely to report multiple concussions. So again it seems that at least if had the APOE promoter gene there is two studies to suggest that that may increase your risk of sustaining a concussion.

A more recent study by Terrell and colleagues, they again looked at APOEe4, APOE promoter gene and tau in a prospective study and they didn’t find any association between these genetic polymorphisms and concussion risk.
Now this is - now we’ll move more towards chronic brain injury and chronic traumatic encephalopathy or the cumulative effects of concussion. This is a study we did almost 15 years ago where we looked at APOE in a group of boxers. So we had a group of 30 boxers that we evaluated, some were still active, some were retired. And I use the term CTBI, chronic traumatic brain injury to be just to state that these are not boxers that necessarily had CTE, but these are just individuals that might have some neurological impairment from their exposure to boxing. And what we did, we devised a brain injury scale where we actually could quantify the amount of impairment. So this scale went from 0 to 9, so the lower the number the less neurological impairment you had and when we looked at the neurological impairment among 4 groups what we did was we stratified by APOEe4 genotype and exposure. Now for most of you, you probably are aware that one of the biggest risk factors at least in the boxing that has been documented probably one of the biggest risk factors for chronic traumatic encephalopathy and probably chronic traumatic brain injury is exposure. So and it makes sense the longer you are exposed to a sport the more likely you are going to have neurological impairment. So being that exposure was a risk factor we thought it was important to stratify according to exposure and the e4 allele. And this is a good interesting point because it highlights the interaction between genetics and our environment. And basically as I look at it there is two things in life, it’s the genes that you are born with and there is the environment and how you interact with that environment. So what happens here is if – this is H stands for high exposure, and this is the e4 allele, so if you had – if you didn’t have high exposure so if you had low exposure and it didn’t matter whether you had the e4 allele or not, the amount of neurological impairment in the group was the same. If you had high exposure and didn’t have the gene then you had moderate neurological injury. However, if you had the high exposure and the gene then you had
more neurological impairment so this was suggestive of interaction between exposure and having the gene. And this was statistically significant.

Then we also looked at severity, and the frequency of having the gene. In the severe group all of them actually had at least one copy of the e4 allele. The normals in this group had, 18% had copies of the e4 allele. And as I mentioned in the general population it’s probably about 25% is the carrier rate for the e4 allele.

We repeated this study in a professional football team, this is the team, the New York Giants. We did neurocognitive testing on them and looked APOE genotype and in this situation what we did was we didn’t have good exposure data in terms of actually how many years they were playing so we used age as a proxy for exposure. So what we did, we stratified according to age and e4 allele and again we found a similar interaction, those older football players, these are all active football players. The active football players the older ones who had the e4 allele tended to have more cognitive impairment than those that didn’t have the allele and those with the allele and low exposure. So again it seems like there is an interaction between genetics and environment with exposure being the environmental factor.

Other studies that have looked at APOE, Ann McKee in her initial publication where she reviewed 48 cases of CTE in the literature and added 3 cases of her own there were 10 cases out that series where they actually reported APOE genotype and 50% carried at least 1 e4 allele, and one case was a 4/4, homozygous. Again as I mentioned in the general population it’s about 25% is the carrier rate.
So at least in this study it was suggested that perhaps e4 may be more highly represented in this group.

However Amalu in his study which again these are small numbers, he had 7 CTE positive athletes that had APOE genotyping and he didn’t find – did not find an increase association of e4 allele and that was only 29%. So that’s similar to what we see in the general population.

And more recently Ann McKee looked at a subset of football players and they didn’t find any difference in the carrier rate of e4 compared with the general population. But if you look at the – her data carefully there may be a suggestion, I think this needs to be worked out more, that if you stratify by age it seems like perhaps the older e4 cases that she looked at may have more severe disease and more tau deposition, more amyloid deposition. I think this needs to be worked out. I guess this morning what was also interesting, Dr. Maroon mentioned that in the study they just did. They didn’t find any association between APOEe4 and CTE. So I think that the jury is still out in regards to e4 and its possible influence on CTE.

In concluding, I’m just going to mention that neuroimaging can play an important role in terms of being a biomarker, there is various techniques that may be available as you heard from my previous speaker, DTI and this high definition fiber tracking could probably serve as a biomarker for traumatic brain injury, spec scan, PET scans may serve as a biomarker. So for example if we are ever able to develop a tau ligand where we can actually visualize tau in the brain perhaps that might help us make the antemortem diagnosis of CTE. Another way that PET can be used, I have been using
amyloid PET scans in a few cases to rule out amyloid type pathology. Probably about 50% of CTE cases will have some type of amyloid deposition. So you don’t need amyloid to have CTE, but if you don’t see any amyloid that may at least rule out an Alzheimer’s type picture and maybe provide more evidence that this could be CTE.

So to conclude to date there is no reliable biomarker that can detect sports concussion. I think the plasma tau may be promising but I think that study needs to be duplicated. APOE genotype may influence outcome following general non-athletic traumatic brain injury, but it’s role in the recovery from sports related concussion and influence on the development of chronic traumatic brain injury needs to be further developed.

And in regards to genes I think it’s important that we look at the interaction between genetic predisposition and the environment. I think that’s important. And when assessing the influence of a particular gene it’s important that you look at the proper function of that gene and assess that variable.

With that I will conclude. Thank you.