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A meta-analysis about the influence of homozygosity in ADH, ALDH, GABA-A receptor and Dopamine receptor D2 genes on the risk of alcohol dependence

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Contents

1) Introduction

Alcohol dependence as a common disease all over the world and especially in the Western society is influenced by several factors (Bosron et al., 1989). The results of alcohol dependence are massive and not just limited on the organic damage but also affecting social behaviour and psychological health. Therefore many different studies paid attention to the pathogenesis of developing alcohlism as well as to the impacts on patients, seen from the internistic and the intersocial perspective. This still remains subject of actual research programs because of the complicated metabolism and effects of ethanol on the human organism. So far it became clear that long-term ethanol consumption combined with a steadily increasing amount of consumed ethanol plays an important role in progressing an addiction (Konishi et al., 2003). Moreover the drinking behaviour of people can be influenced by risk factors that lead to the consumption of ethanol, such as peer pressure, life crisis or social exclusion which refers to the environmental significance for alcohol dependence (Bosron et al., 1989). A quite similar importance for understanding the complexity of this multifactorial illness has got the analysis of the patients' genetics, which became a large field in latest research programs. To evaluate the complex results of different studies, we worked out a meta-analysis about homozygous SNP carriers that have to cope with altered proteins playing a role in the human organism's reaction to alcohol consumption in comparison to carriers of the wildtype alleles. What is more we evaluated the meaning for their behaviour concerning regular alcohol consumption in regard of their genotype. With our meta-analysis we could make the comparison between these studies more bearable and enable people to identify potential genetic predispositions associated with the progress of alcohol dependence. Although the identification of problematic chromosomal regions influencing the progress of alcoholism isn't finished at all, there is strong evidence provided for several polymorphisms (Edenberg et al., 2006). Quite often they consist in single nucleotide polymorphisms (SNPs), which emphasizes that even small genetic differences cause big clinical effects, as we also know from lots of other diseases such as mucoviscidosis for example (Smerieri et al., 2014). The modified alleles cause variable proteins that show different interaction with ethanol and might lead to a higher risk of becoming an alcoholic,

for example by altered reactions to consumption, different agreeability or withdrawal symptoms (Enoch et al., 2008). The homozygous carriers of mutations theoretically show a bigger difference from the wildtype than the heterozygous ones. This is why we investigated our meta-analysis only with homozygous genotypes. Thus our findings should deliver clear differences between the homozygous wildtype allele carriers and the mutated allele carriers that also might own validity in heterozygous individuals in a probably lower stamping dependent on the way of inheritance. The majority of the studies we analyzed especially focused on the following proteins which are the main objects of our meta-analysis as well:

1. **ADH Genes:**

   The alcohol dehydrogenase (ADH) is the ethanol metabolizing enzyme. This redoxreaction leads to the creation of acetaldehyde, a neuro-toxic substance (Matsuo et al., 2007). The ADH is located in mainly the liver cells and in a smaller amount in the stomach. The enzyme plays an outsize role in the metabolism of ethanol and is encoded by genes on chromosome 4 (Edenberg et al., 2006). To be able to oxidize ethanol, the alcohol dehydrogenase is dependent on the availability of the cofactors NAD^+ and Zn^{2+}(Choi et al., 2005). It is obvious that examining the enzyme's activity is inalienable when considering the genetics of alcohol dependence. The different polymorphisms of the genes encoding for the ADH isoenzymes (ADH1A, ADH1B, ADH1C, ADH2, ADH3, ADH4, ADH5, ADH6, ADH7) have been subject of several case-control studies (Shen et al., 1997) on which results we broach the issue on below. Especially the class I isoenzymes are supposed to be important for the metabolism of ethanol. The speed of the alcohol degradation is also crucial for the withdrawal symptoms as it may enlarge the time the body is under the influence of acetaldehyde (Thomasson et al., 1991). (The same reaction can be catalysed by the MEOS – mitochondrial ethanol oxidizing system – that can be induced by regular drinking (Hubacek et al., 2014). This leads to the resistence towards symptoms such as motor incoordination for example but reveals the problem of an increased level of

acetaldehyde that is not recogniable and that leads to cell damage especially in neuronal areas.)

2. ALDH Genes:

The acetaldehyde dehydrogenase (ALDH) is responsible for the oxidation of acetaldehyde, the molecule which is mainly causing hangover symptoms after an excessive alcohol consumption and particularly affecting the neuronal system (Goedde et al., 1992). To process the degradation of acetaldehyde, the enzyme needs $NAD(P)^+$ as a hydrogen acceptor. There exist 9 major gene families coding for isoenzymes such as ALDH1, ALDH2, ALDH3-ALDH9 (Ehlers et al., 2012). Above all chromosome 12 contains notable genetic variation according to ALDH genes, particularly for the ALDH2 gene, so that this chromosomal area has an outstanding role analyzing the effects of mutations on the metabolism of acetaldehyde (Husemoen et al., 2008). The ALDH2 has got a low Km and is located in the mitochondria. It is the isoenzyme with the biggest importance for the oxidation of acetaldehyde (Hurley et al., 2012) and its deficiency influences the occurrence of aversive symptoms which also regulate the risk of developing an alcohol addiction.

3. GABA-A Receptor Genes:

Several genetic polymorphisms are identified for this pentameric ion channel that is assembled by α, β, γ, δ, ε and ρ subunits (Buck et al., 1996). The loci of the relevant alleles are limited on chromosome 4, 5 and 15 that provide genetic information for the different subunits. Although there is really good evidence for the importance of GABA reception for the metabolism of ethanol and for the fact that alcohol leads to a higher rate of activity of this receptor that causes the decreased central nerval alertness, the precise mechanisms of GABA reception involvement in the pathogenesis of alcoholism remains unknown and are characterized by different reactions in different neuronal areas (Covault et al., 2008). These different reactions caused by diverse neurotransmitter concentration alterations and different receptor

efficiencies between the brain regions are dependent on the composition of GABA-A receptors which is influenced by several genetic polymorphisms. In particular the alleles of the α2-subunit were taken into consideration to differ between alcoholic patients and healthy controls, thus being suspicious to increase the individual risk of becoming alcohol dependent by SNPs in that gene (Enoch et al., 2008). What is more the importance of the GABA-A receptors on behavioural effects like motor incoordination, anxiolysis, sedation, withdrawal signs, that are all closely linked with alcohol consumption, are stated (Buck et al., 1996).

4. Dopamine Receptor D2 Genes:

The fact that dopamine plays a huge role in neuronal systems is known at the latest since the understanding of Parkinson's disease (Wu et al., 2014). Furthermore the metabolism of dopamine and especially its receptor activity has impact on other physiological and pathological pathways throughout the central nervous system that are also related to the symptoms of alcohol consumption (Bhaskar et al., 2010). Thus the genetic information encoding 5 different types of dopamine receptors (DRD1-DRD5) is regarded to be of major importance for the understanding of the neuronal mechanisms of alcohol. Several alleles particularly in the dopamine receptor D2 (DRD2) have been focused and linked with a predisposition for or a protection from alcoholism (Joe et al., 2008). The TaqIA polymorphisms for example, that lies 10 kb downstream of the DRD2 and contains gentic information for a kinase gene, ANKK1 (ankyrin repeat and kinase domain containing 1), is associated with the density of DRD2 in the human brain (Singh et al., 2013). The receptor density is responsible for interindividual effects of ethanol. Participants with a low density of receptors in the central reward pathway such as the nucleus accumbens or amydala for example require higher amounts of alcohol to achieve benefit feelings or positive emotional effects from ethanol consumption in comparison to those having a higher density of DRD2 receptors (Noble et al., 2003). Additionaly the -141C Ins/Del polymorphism of an intronic region of the DRD2 gene is also considered to influence the density of DRD2 (Ishiguro et al., 1998). That's why we investigated the data especially for these polymorphisms of the DRD2 which are all together located on chromosome 11.

2) Materials & Methods

Literature search

We searched in Pubmed for relevant studies that were focusing on the genetic background having influence on the likeliness on becoming alcohol dependent. We used the following terms: “adh alcohol dependence”, “aldh alcohol dependence”, “gaba receptor alcohol dependence”, “dopamine d2 receptor alcohol dependence”, “genetics of alcohol dependence”, “adh SNPs alcohol”, “aldh SNPs alcohol”, “gaba receptor SNPs alcohol”, “dopamine d2 receptor SNPs alcohol”, “adh polymorphisms”, “aldh polymorphisms”, “gaba receptor polymorphisms”, “dopamine d2 receptor polymorphisms”, “adh1b”, “adh1b*1 alcohol”, “adh1b*2 alcohol”, “adh1c*1 alcohol”, “adh1c*2 alcohol”, “aldh2”, “aldh2*1 alcohol”, “aldh2*1 alcohol”, “gabraa2 alcohol”, “drd2”, “-141C alcohol”, “TaqIA alcohol dependence”,. We also had a look after the references in the eligible articles or textbooks for more studies that we could include in our meta-analysis.

Inclusion or exclusion criteria

The studies we included in our meta-analysis met the following criteria: (a) case-control studies, nested case-control studies, follow-up studies embracing cases and controls, cohort studies dividing the participants into cases and controls after genotyping them and referring to the answers of the performed interviews or questionnaires that were all focusing on the associations between ADH, ALDH, GABA-A receptors and DRD2 polymorphisms and alcohol dependence; (b) the patients with psychiatric disorders should meet DSM criteria that were present for the time of the study's publication; (c) the minimum number of cases in included studies should be bigger than 40, except from the studies that were divided into several ethnic subgroups, then we calculated the risk of becoming alcohol dependent separately for each ethnic subgroup plus the whole study’s population; (d) the data on the genotype and

allele frequencies must be sufficient and comprehensible; (e) papers had to be published in reviewed journals.

Studies were excluded when they were: (a) not case-control studies, not nested case-control studies, not follow-up studies embracing cases and controls, not cohort studies dividing the participants into cases and controls after genotyping them and referring to the answers of the performed interviews or questionnaires about the associations between ADH, ALDH, GABA-A receptors and DRD2 polymorphisms and alcoholism; (b) based on incomplete data; (c) dealing with group sizes that were smaller than 40 in the whole study, except from a division of the case or control groups into ethnic subgroups; (d) duplicate publications of data from the same studies; (e) meta-analyses, editorial articles or abstracts without a complete article.

Data extraction

We extracted the data by choosing the studies from Pubmed randomly and thus avoided selection bias. For the majority of the studies, the following characteristics were collected: the first author, year of publication, language, study design, number of subjects, source of cases and controls, genotype methods, allele frequencies, genotype frequencies and evidence of Hardy-Weinberg Equilibrium (HWE) in controls. Inconsistent data was reexamined by working carefully through the whole text and when indicated excluded from the meta-analysis afterwards.

Quality assessment of the included studies

The quality of the studies had been analysed by carefully reading through any study included, paying attention to the fact if they were fulfilling our inclusion criteria. Therefore some studies had to be excluded later on because the study designs offered some deficiencies in processing examinations of the participants or mingling alcohol dependence together with other psychiatric and somatic disorders without being completely transparent. Moreover we took care that we just included studies that showed appearance of our inclusion criteria such

as adequate group sizes as well as reliable and valid study designs. So we summed up the number of cases and controls in individual tables for each SNP. In general we considered retrospective, controlled, matched-pairs designs in case-control studies and follow-up studies or cohort studies with a high compliance as the characteristics of a valide and reliable study design.

Statistical analysis

Odds Ratios (ORs) and 95% Confidence Intervals (CIs) were calculated for the intensity of the association between the genetic polymorphisms of the ADH, ALDH, GABR and DRD2 genes with the likelihood of becoming alcohol dependent. The statistical significance of the Odds Ratios was evaluated through the Z test. Variations between studies were estimated by using Cochran's Q statistic. A P-Value of <0,05 was considered a statistically significant inter-study heterogeneity. We used I^2-test that can also indicate heterogeneity between two studies when ranging levels >50%. Random effects model (DerSimonian Laird method) was conducted when heterogeneity between two studies was given (Q-test: P<0,05 or I^2>50%). Otherwise the fixed effects model (Mantel–Haenszel method) was processed. Moreover we examined some inter-ethnic differences when enough studies were provided dealing with a certain ethnic group and the polymorphism. We compared the significant results of the homozygous genotype frequencies in a Forest Plot for each SNP then being able to interpret ratios of the given results. An OR>1 indicates a bringing forward effect for alcoholism, an OR<1 describes a kind of protective impact. The 95% CIs that do not contain the 1 describe significant results given by the studies. Minor allele frequencies for both cases and controls have been calculated as well as the controls were examined for a deviation from Hardy Weinberg Equilibrium by using chi-squared analysis. All analyses were calculated using the "StatsDirect Statistical Software" Version 3.0.97 (StatsDirect.Ltd, Tidestone Technologies Inc., GB).

Sensitivity analysis

To process the sensitivity analysis for the studies included in our meta-analysis, we checked if the studies' results lay in statistical spread or if the outcome of the data given is influenced by publication bias. Therefore we used a funnel plot to clarify whether publication bias exists. A clear deviation from the symmetry assumption indicated publication bias (Egger's regression test: P<0,05). In case of publication bias, we excluded studies that were potentially responsible for the deviation from statistical spread from a second analysis if their study designs deviated from optimal designs such as retrospective, controlled, matched-pairs designs in case-control studies or controlled follow-up studies with a high compliance and thus not fulfilling our inclusion criteria in an adequate way. As some studies didn't show the characteristics of study designs that deliver reliable and valid data, which was indicated by the standard deviation being very large or the group size very small shown by the size of the 95% confidence interval, we excluded those studies together with the ones not meeting HWE in the control group from a second meta-analysis hence processing a separation of valid, reliable study designs from the ones causing bias. So we performed the chi-square test to identify a lack of meeting of Hardy Weinberg Equilibrium (p<0,05) in the control sample and considered the deviance from HWE in the controls a reason to create a second forest plot for the relative SNP to be sure of genotypic consistency between the generations. Thus we avoided disturbing factors influencing the studies' outcome apart from genetic polymorphisms in an average population. Additionally we considered the results of studies with a confidence interval bigger than 8 as not being significant. So we also excluded them from a second meta-analysis about the regarded SNP. The sensitivity analysis we processed enabled us to differ between good and bad study designs retrospectively. If we indicated publication bias, we tried to handle good study designs apart from bad ones in another statistical analysis. Thus, if possible, we could achieve clearer results for associations between the SNPs and the development of alcoholism after we left out the results of the studies that were responsible for publication bias. In these cases it was reasonable to create a second forest plot for the results of the studies that we still included after performing our sensitivity analysis because of the fact that the left-out ones delivered possibly adulterated data. Afterwards we had a look if we got rid off the publication bias by producing a second sensitivity analysis in another funnel plot. Consequently we got rid of studies' results that are

not just dependent on the genotypic distribution of the participants but also on other disturbing factors, such as the environment, having influence on the drinking behaviour and thus blurring our results. All analyses were calculated using the "StatsDirect Statistical Software" Version 3.0.97 (StatsDirect.Ltd, Tidestone Technologies Inc., GB).

PRISMA 2009 Flow Diagram

We used the PRISMA 2009 Flow Diagram (Moher et al., 2009) to demonstrate how many studies we could include in our meta-analysis either in the quantitative synthesis or the qualitative synthesis and how many studies, that we identified, were not suitable to be included in our statistical analysis because of not meeting our inclusion criteria or being duplicates from studies we already examined or delivering the data in an idiosyncratic way that made a statistical analysis impossible. After the screening of the records we identified to be useful for our work, we assessed 212 full-text articles for their eligibility and finally included 158 studies into our analysis of which we could work with 52 articles' quantitative data. We illustrated the process of collecting the final number of studies included from the original 374 identified articles in the following Figure:

PRISMA 2009 Flow Diagram

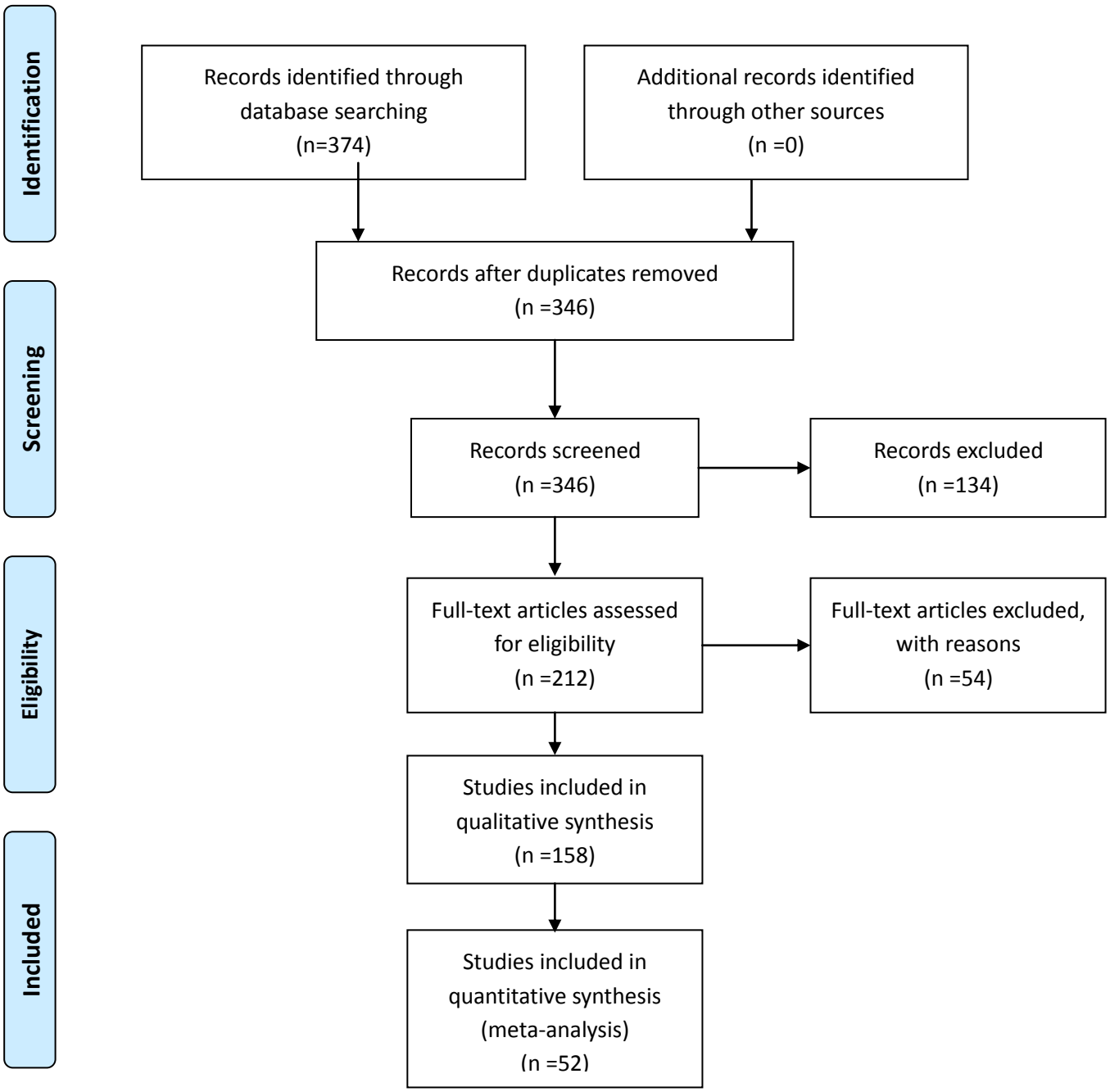


Figure1.0

PRISMA 2009 Flow Diagram (Moher et al., 2009) showing the number of the studies we identified, screened, tested for their eligibility, excluded and included in our meta-analysis.

3) Results

We were looking for the best examined single nucleotide polymorphisms of the genes of the ADH, ALDH, GABA-A receptor and DRD2 and collected the data in **Table 1.0**. So we could just include those polymorphisms in our meta-analysis that were examined by several different authors for having an association with the development of alcohol dependence to make sure that our analysis could achieve significant results. To be able to differ between the meaning of the regareded SNPs for the progress of alcohol dependence in certain ethnic groups, we also illustrated the ethnicities of the subjects the articles were dealing with together with the whole group sizes of cases and controls in the Table below. In so doing we tried to work out if inter-ethnic differences exist in either genotype frequencies or the meaning of being carrier of a specific genotype for the individual protection from or the individual risk of becoming alcohol dependent.

Author	**Year**	**n[a] (cases)**	**n[a] (controls)**	**Ethnicity of the subjects**	**SNPs[b]**
Geijer	1994	74	81	Caucasian	DRD2 TaqIA
Shen	1997	52	48	Asian (China)	ALDH2
Shen	1997	55	50	Asian (Korea)	ALDH2
Ishiguro	1998	209	152	Asian	DRD2 TaqIA/DRD2 -141C
Chen	1999	340	545	Asian	ALDH2
Lee	1999	128	85	Asian	DRD2 TaqIA

Gelernter	1999	160	136	Caucasian	DRD2 TaqIA/DRD2 -141C
Sander	1999	310	196	Caucasian	DRD2 TaqIA/DRD2 -141C
Bau	2000	115	114	Brazilian	DRD2 TaqIA
Shaikh	2001	50	53	Asian	DRD2 TaqIA
Lu	2001	97	85	Asian	DRD2 TaqIA
Pastorelli	2001	60	64	Caucasian	DRD2 TaqIA
Chen	2001	228	215	Asian, Aborignal	DRD2 TaqIA/DRD2 -141C
Shaikh	2001	50	53	Asian	DRD2 TaqIA
Chao	2003	361	280	Asian	ALDH2
Huang	2004	184	281	Asian	ADH1B
Konishi	2004	200	251	Mexican American	ADH1B/ADH1C/ALDH2/ DRD2 TaqIA/DRD2 -141C
Foley	2004	87	109	Caucasian	DRD2 TaqIA
Lappalainen	2005	113	100	Caucasian	GABRA2
Luo	2005	200	251	Mexican American	DRD2 TaqIA/DRD2 -141C
Choi	2005	99	225	Asian	ADH1B/ADH1C
Fehr	2006	257	88	Caucasian	GABRA2
Drgon	2006	415	239	African American, Caucasian	GABRA2
Berggren	2006	357	842	Caucasian	DRD2 TaqIA
Luo	2007	334	317	European American	ADH1B/ADH1C
Luo	2007	102	482	African American	ADH1B/ADH1C

Tseng	2007	100	98	Asian	ALDH2
Soyka	2007	316	295	Caucasian	GABRA2
Huang	2007	108	201	Asian	DRD2 TaqIA
Wang	2007	73	158	Asian	DRD2 TaqIA
Covault	2008	372	535	European American, African American	GABRA2
Samochowiec	2008	122	150	Caucasian	DRD2 TaqIA/DRD2 -141C
Du	2009	365	338	Mexican American	DRD2 -141C
Kraschewski	2009	360	368	Caucasian	DRD2 TaqIA/DRD2 -141C
Khan	2010	325	395	Asian	ADH1B/ADH1C
Cichoz-Lach	2010	204	172	Caucasian	ADH1B/ADH1C
Tan	2010	78	104	Asian (China)	ADH1B/ALDH2
Tan	2010	104	80	Asian (India)	ADH1B/ALDH2
Shin	2010	68	232	Asian	ALDH2
Bierut	2010	1897	1932	European American, African American	GABRA2
Sakai	2010	371	185	Caucasian, Hispanic	GABRA2
Prasad	2010	90	60	Asian	DRD2 TaqIA/DRD2 -141C
Bhaskar	2010	81	115	Asian	DRD2 TaqIA
Kovanen	2010	512	511	Caucasian	DRD2 TaqIA
Lu	2010	133	244	Asian	DRD2 TaqIA
Guo	2010	383	350	Asian	ADH1B/ALDH2

Tóth	2011	241	666	Caucasian	ADH1B/ADH1C
Aktas	2012	75	100	Turkish	ADH1B/ADH1C
Ehlers	2012	209	313	Mexican American	ADH1B/ADH1C/ALDH2
Ehlers	2012	434	357	Native American	ADH1B/ADH1C/ALDH2
Kortunay	2012	90	100	Turkish	ADH1C
Ittiwut	2012	380	253	African American	GABRA2
Schellekens	2012	119	99	Caucasian	DRD2 TaqIA
Suraj Singh	2013	129	286	Asian	DRD2 TaqIA/DRD2 -141C
Lee	2013	189	110	Asian	DRD2 TaqIA/DRD2 -141C
Bjerregaard	2014	531	3631	Inuit (Asian)	ADH1B/ADH1C/ALDH2
Bjerregaard	2014	9080	3631	Caucasian	ADH1B/ADH1C/ALDH2
Bjerregaard	2014	69	3631	Yupik Escimo, Alaska	ADH1B/ADH1C/ALDH2

[a]total number of cases and controls in the relative study

[b]regarded SNPs in the respective study

Table 1.0
Characteristics of the studies included in our meta-analysis given by author, year of publication, number of cases, number of controls, ethnicities the relative study is dealing with and SNPs that are considered to play a significant role in the development of alcohol dependence.

3.1) ADH genes

As the enzyme that metabolizes ethanol, the alcohol dehydrogenase (ADH) obviously is of outstanding importance for the ethanol consumption and the development of an addiction. It catalyzes the oxidation of ethanol to acetaldehyde and water using the coenzyme NAD^+ as a hydrogen acceptor. The negative effects of acetaldehyde are responsible for the typical hangover symptoms and mostly of neuronal nature (Edenberg et al., 2006). In those histoids a certain concentration of acetaldehyde remains according to the alcohol consumption over a longer or shorter time after ethanol consumption. The concentration of the neurotoxic acetaldehyde and the time ethanol is available in the blood differ between individuals because of the different efficiencies of the eliminating enzymes caused by genetic predispositions that lead to different isoenzymes and thus different Kms and times of alcohol degradation. The biggest amount of the ADH can be found in the cytosol of the liver cells but some of the isoenzymes are also located in the upper gastro-intestinal tract and oxidize ethanol in high concentrations (Park et al., 2013). Genetic variations causing different pharmacokinetics of this enzyme therefore play an important role concerning the pathogenesis of alcoholism (Edenberg et al., 2006). Considering the effects of acetaldehyde, the product of the alcohol dehydrogenase, it becomes clear that the higher the metabolism of ethanol the stronger are the hangover symptoms and the more unlikely becomes a permanent consumption of alcohol. So the mutations leading to an increased risk for alcohol dependence actually should lead to an isoenzyme of the ADH that metabolizes ethanol more slowly and hence reduces negative effects of ethanol consumption (Matsuo et al., 2007). Thus the positive short-term effects of ethanol remain for a longer time which makes the repeated consumption of alcohol more likely. It becomes obvious that regardless from the way of inheritage particularly homozygous carriers either of the wildtype or the mutation differ between their enzyme activity. Genetic information causing the creation of different subunits of the alcohol dehydrogenase are located on chromosome 4q22-q23. This area is responsible for the biggest part of the genetic information of the isoenzymes such as ADH5, ADH4, ADH6, ADH1A, ADH1B, ADH1C and ADH7 (Shen et al., 1997). Variable mutations in that chromosomal area lead to diverse effects influencing the efficiency of the isoenzymes. More exactly, it has been described, that 13 of the top 30 SNPs having impact on the

metabolism of ethanol by the creation of different subunits of the ADH, are located on chromosome 4q22-q23 (Park et al., 2013). This is also the reason why our meta-analysis basically focussed on the two homozygous genotypes of that chromosomal area.

The studies we analyzed mostly examined one specific ethnic group. We found different inter-ethnic results which emphasize the importance of the ethnic background for the existence of polymorphisms in the ADH genes. So there are people who are more likely to develop alcoholism because of the existence of genetic variants in comparison to people from another part of the world. Moreover there is quite good evidence for a link between alcohol dependence and certain homozygous genotypes, while other polymorphisms have not been identified to have an association with AD.

One genetic variation concerning the ADH1B gene (rs1229984), which is, as most of the other mutations, located on chromosome 4, seems to be of major importance for repeated alcohol consumption as there are many studies with good results for this mutation. The most frequent change in this genetic sequence consists of a switch from adenine to guanine (A→G) leading to an amino acid exchange of arginine to histidine (ADH1B Arg48His) which changes the ADH1B*1/1 (Arg/Arg) genotype to the ADH1B*2/2 (His/His) genotype in the case of two mutated alleles (Aktas et al., 2012). Dependent on the amino acid there results a slow isoenzyme from Arg/Arg genotype and a fast isoenzyme from His/His genotype. So this switch leads to a protection from alcoholism by creating a faster isoenzyme that leads to more negative side effects by enlarging the amount acetaldehyde circulating in the participant's blood. Additionally the shortened time of ethanol circulation reduces positive feelings such as low central nervous alertness and activation of the reward system in the brain (Lu et al., 2001). These effects are the biggest in homozygous carriers for the ADH1B*2/2 genotype which is widely spread in most of the population. This is why the SNP leading to the amino acid Arg is associated with alcohol dependence, in particular within the homozygous genotypes.

Another study examined 106 people around Seoul with alcohol dependent illneses but without psychiatric problems and 246 controls. They worked out a cross-fostering study in which they observed a higher twin pair concordance in monozygot twins than in dizygot twins (Choi et al., 2005). To genotype these participants they used the standard methods for extracting DNA and after sequencing of the ADH1B gene as well as the ADH1C gene, they

revealed 36 variants of these sequences which mainly consisted of the ADH1B (A→G; SNP ADH1B*1 leading to Arg) showing an association with alcohol dependence and ADH1C (A→G; SNP ADH1C*1 leading to Ile) also giving evidence for an association with alcoholism. The OR for the homozygous ADH1B*1/1 carriers was 13,1. So this seems to represent the high-risk genotype in that Korean population. ADH1C*1/1 seemed to act protectively against alcoholism (OR=0,56). The potential high-risk SNP ADH1C*2 leading to the amino acid Val couldn't be found in homozygosity for the controls. The heterozygous genotypes' risk for alcohol dependence lied between the one of the homozygous carriers.

Edenberg et al. (2006) also discovered an association between the ADH1B variants and alcoholism. The ADH1B*2 allele seemed to be protective against the development of alcohol dependence. Furthermore the replacement of Arg in position 48 with His in the ADH1B*2 gene leads to a subunit which has a 40-fold higher Vmax than the subunit encoded by the ADH1B*1 allele. Thus the enzyme works faster and the concentration of acetaldehyde will be higher in comparison to the slow oxidation and this should lead to a lower risk for a developing alcoholism because of increased negative effects caused by the product of the alcohol dehydrogenase.

To evaluate the importance of SNPs in the ADH gene clusters, the study by Ehlers et al. (2012) processed an interview with the Semi-Sturctured Assessment for the Genetics of Alcoholism (SSAGA), which is a polydiagnostic psychiatric interview that has undergone reliability and validity testing, and took blood samples from each participant of a group of 924 Native Americans and 522 Mexican Americans. They wanted to clarify, if a clear difference in allele frequencies exists between cases and controls of different ethnic groups. So they compared the results of a community sample of Native Americans living in reservations with those of Mexican Americans living in the same county (San Diego County, groups of at least 20% Hispanic heritage residents). The presence of heterozygous allele carriers for the rapidly metabolizing isoenzyme encoded by the ADH1B*2 allele achieved 7% in the Native American population and 13 % in the Mexican American population. But there were nearly no subjects with homozygous genotypes (ADH1B*2/2) which made a statistical analysis for this group of participants impossible. So an association between the ADH1B*2 allele and a protection from alcoholism could only be proven in heterozygous Mexican American participants, while the ADH1B*3 allele, an allele that hasn`t been described by many other studies, was

considered being protective against the development of alcohol dependence in Native American populations too. Its prevalence was quite low though (4% of that population were carriers for that allele). The study didn`t confirm an association between ADH1C mutations and alcohol dependence (for example for Mexican Americans: ADH1C*1/1: OR=0,88; ADH1C*2/2: OR=0,92). Thus their results differ from other studies examining similar ethnic groups. For example in a Mexican American population there was a clear linkage between ADH1B*1/1 and ADH1C*2/2, and thus the slow metabolizing isoenzymes, and the risk of becoming alcohol dependent (Konishi et al., 2004). Still in some populations there are just not enough homozygous individuals to be able to make clear statements about the meaning of the SNP. What is more this makes clear that the numbers of participants have to be high enough to create reliable and valid results on that topic as the prevalence is very low. The study by Ehlers et al. (2012) also conducted departures from the Hardy-Weinberg equilibrium (HWE) for alcoholics and non-alcoholics as a quality check of the data given by the microbiological tests. After all they analysed the achieved data for frequency of the ADH1B, ADH1C as well as ALDH2 alleles and the determination of an association between alcoholism and the allelic variants. Ehlers et al. (2012) hypothesized an “unusual metabolism of alcohol” in Native American groups after evaluating the results with view on the little number of ADH1B*2/2 genotypes that one can find much more frequently among population of another ethnic backgrounds. In other ethnic groups this is even the most frequent genotype (Lu et al., 2001) and the base adenine leading to the amino acid arginine is quite rare. As a critical view on that study it is important to notice that the mean age of the participants of Mexican American background lay only about 23,5 years. That leaves us with the problem that a potential alcohol dependence will just establish in later years and thus the results of that study have to be seen quite critical on that issue.

The next study focused again on the role of ADH1B Arg48His (rs1229984) as well as the role of ADH1C Ile350Val (rs698) for developing alcoholism in a Turkish population (Aktas et al., 2012). They took into consideration the information of 100 healthy volunteers and 75 patients admitted to the Ege University Alcohol Dependence Unit. While the microbiological (restriction fragment length polymorphism), demographic, statistical (OR, Chi-square test, Hardy-Weinberg equilibrium) and psychological analysis didn`t show any connection between alcoholism and the ADH1C (Ile350Val) polymorphisms, the ADH1B (Arg48His) polymorphisms seemed to lead to a significant increase in alcohol dependence when being

carrier of two adenines (ADH1B*1/1) which was highly prevalent among the patients. Again the reason why this genotype causes these problems, lies in the fact that a slow isoenzyme results from the ADH1B*1/1 genotype and as we already mentioned above, this polymorphism leads to a different rate of ethanol elimination and thus another concentration of acetaldehyde altering the risk of becoming an alcoholic (OR=4,0; 95% CI:1,42-11,27). The confidence interval is quite large which is caused by the small amount of cases being carrier for the ADH1B*1/2 and ADH1B*2/2 allelic combinations. Another study analysed the combined and single effects of ADH1B (rs 1229984, rs2066702) and ADH1C (rs1693482, rs698) alleles on alcohol dependence and chronic liver diseases (Tóth et al., 2011). They used the data from 241 cases with chronic liver diseases and 666 randomly selected controls without any liver disease and were processing a questionnaire and a genotyping analysis as well. Mainly four SNPs leading to the following amino acid switches were associated with alcoholism: Arg48His, Arg370Cys in ADH1B and Arg272Glu, Ile350Val in ADH1C. Especially the Arg48His mutation in the ADH1B gene cluster illustrates a SNP that has been examined by some other authors. In particular combined with the wild type of the ADH1C allele (ADH1C*1), the ADH1B*2 allele (His) showed a protective effect against chronic liver diseases and alcoholism. ADH1C polymorphisms were showing no association with the disease of alcoholism (ADH1C*1/1: OR=1,03; ADH1C*2/2: OR=1,07). There was no subject that is homozygous for the ADH1B*2/2 genotype in the patients' group. This leaves us with the problem of not being able to use the data for a forest plot but stresses the protective impact of the SNP against alcohol dependence as no alcohol dependent participant of this study carries the homozygous variant of the ADH1B*2 allele.

Revealing more numbers in Asian populations, another study by Tan et al. (2010) gave results that drew an association between the high-risk genotype ADH1B*1/1 and alcohol dependence (Chinese individuals: OR=3,93; Indian individuals: OR=1,85). They could also find more ADH1B*2 subjects in the control sample compared to other studies. This control sample consisted of 184 screened persons from Singapore. The cases were 182 Chinese and Indian men and women who underwent treatment for alcohol dependence and performed the AUDIT (alcohol use disorder identification test). The mean age of the cases was about 45 years and the one of the controls at about 34 years. In the end after comparing their data with studies dealing with Caucasians the main conclusion was composed of the fact that the

protective alleles were much more frequent in Asian than in European populations for both, ADH and ALDH polymorphisms (Tan et al., 2010).

As alcoholism is socialised with other psychiatric disorders many times, both can be examined at the same time when analysing the genetics of clinically conspicuous patients. Therefore the SNPs of ADH and ALDH had been tested for an association with alcohol dependence and anxiety depressions but also with alcohol dependence only (Huang et al., 2004). What is more the role of the dopamine receptor D2 had been examined and related to mutations of the two enzymes. Therefore 184 Han Chinese subjects and 281 controls were collected for that investigation. While the DRD2 receptor did not show any linkage to psychiatric diseases and alcoholism, the alcohol dehydrogenase did. They did not just show association of the ADH genotypes with alcoholism in an Asian population (ADH1B*1/1: OR=7,27; 95% CI: 3,9-14,0; ADH1B*2/2: OR=0,19; 95% CI: 0,12-0,29), which was already reported by other studies, but they have also been connected with alcoholics with anxiety and depression.

The ADH1B*2 isoenzyme, that has a 100 times higher catalytic activity for ethanol oxidation than the ADH1B*1 isoenzyme (Yoshida et al., 1981), couldn't be found in any of the populations by a study dealing with participants from Greenland (Inuit), Denmark (European) and Yupik Escimos from Alaska (Bjerregaard et al., 2014). They tried to investigate the meaning of ADH1B (Arg48His), ADH1C (Ile350Val) and ALDH2 (Glu504Lys) polymorphisms for develoing alcoholism. Unfortunately there was no statistical analysis possible for the ADH1B and ALDH2 polymorphisms although they examined large numbers of participants. There weren't just any subjects with the homozygous ADH1B*2/2 or ALDH2*2/2 genotypes. That might explain why alcoholism is a huge problem among the Inuit that originally derived from Siberia in Asia. As we can state in the text below, the ALDH2*2 allele is widely spread among Asian populations compared with other ethnicities thus protecting them from alcoholism by encoding for an isoenzyme that is partly inactive. This leads to the accumulation of acetaldehyde and enlarged hangover symptoms that make regular alcohol consumption more unlikely. This polymorphism is not very frequent among the Inuit individuals which differs them from other Asian ancestries and which might explain why they have a quite high rate of alcohol dependent subjects among their population in comparison with other Asian populations also keeping in mind their environmental circumstances that may also play an

important role. Moreover the fact, that the ADH1B*1/1 genotype is the only one found among the participants, supports the ALDH2*1/1 genotype in representing high-risk polymorphisms according to alcohol dependence. This is another difference to other ethnic groups that benefit from protective allele constellations that might also explain the general problem of quite high amount of alcohol addicted individuals among the population of Danish Europeans, Inuits in Greenland and Yupik Escimos in Alaska. Another Asian population was examined by Guo et al. (2010). They analyzed 383 alcohol dependent cases and 350 unrelated healthy controls for the association of ADH1B and ALDH2 polymorphisms with alcoholism. ALDH2*2/2 and ADH1B*2/2 were both negatively associated with alcohol dependence in this population.

European American and African American subjects had been analysed for the meaning of the ADH1B and ADH1C polymorphisms for the development of alcoholism (Luo et al., 2007). All genotype frequency distributions were in Hardy Weinberg Equilibrium in the controls for both of the examined groups. Clear connection between the high-risk genotype and alcohol dependence was especially given for the ADH1B*1/1 mutation in European American samples. Also other isoenzymes of the ADH had been taken into consideration to be associated with alcoholism in these ethnic groups (ADH5, ADH6, ADH7). In particular the ADH5 polymorphisms showed significant linkage with alcohol dependence. What is more the homozygous ADH1C polymorphisms (ADH1C*1/1 and ADH1C*2/2) did not differ in their effect on the vulnerability to alcoholism as the OR remained similar in both carriers for the high-risk and low-risk genotype in the population of African Americans and European Americans.

Some studies about the SNPs of ADH1B had been excluded from the meta-analysis because there was no control group: Visvanathan et al. (2007), Yokoyana et al. (2013), Yokoyana et al. (2014), Linneberg et al. (2010). Other studies had to be excluded because they were not delivering data about the genotypic distributions: Kuo et al. (2008), Kim et al. (2008), Park et al. (2013), Mulligan et al. (2003), Gizer et al. (2011). The study by Ma et al. (2005) had to be excluded because the participants were divided into numerous tiny regions of China so that there was no data given for a population that was big enough to create significant results.

We collected the data given by the studies dealing with ADH1B*1/1 (adenine/adenine; 48Arg/48Arg) genotypes and summed up the results in **Table 1.1**:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Huang	2004	Taiwan	56	16	0,42	0,27	0,49	7,27 (3,9-14,0)
Konishi	2004	USA	195	228	0,02	0,05	0,06	3,9 (1,42-13,5)
Choi	2005	Korea	46	14	0,42	0,21	0,13	13,1 (6,4-27,5)
Luo, EA	2007	USA	218	215	0,03	0,06	0,35	2,73 (1,24-6,49)
Luo, AA	2007	USA	83	41	0,01	0,02	0,87	13,9 (1,69-646,1)
Khan	2010	India	258	341	0,1	0,08	0,04	0,61 (0,4-0,92)
Cichoz-Lach	2010	Poland	200	152	0,01	0,06	0,63	6,55 (2,13-26,9)
Tan, Chin.	2010	Malaysia	13	5	0,36	0,26	0,3	3,93 (1,24-14,76)
Tan, Indian	2010	Malaysia	46	24	0,34	0,43	0,26	1,85 (0,96-3,6)
Guo	2010	China	315	230	0,09	0,16	0,46	2,41 (1,69-3,46)
Tóth	2011	Hungary	220	554	0,04	0,09	0,15	2,12 (1,28-3,65)
Aktas	2012	Turkey	68	68	0,04	0,12	0,79	4 (1,42-11,27)
Ehlers, MA	2012	USA	119	265	0,03	0,08	0,71	3,07 (1,33-8,29)
Ehlers, NA	2012	USA	408	329	0,06	0,04	0,45	1,33 (0,74-2,42)

Bjerre-gaard, Inuit	2014	Denmark	531	3631	0	0	<0,001	-
Bjerre-gaard, Cauc.	2014	Denmark	8626	3631	0,02	0	<0,001	-
Bjerre-gaard, Yupik	2014	Denmark	69	3631	0	0	<0,001	-

[a]Number of cases being carrier for the ADH1B*1/1 genotype

[b]Number of controls being carrier for the ADH1B*1/1 genotype

[c]Minor allele frequencies for the populations' ADH1B*1/1 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the ADH1B*1/1 genotype and alcohol dependence

Table 1.1

Characteristics of the studies dealing with carriers for the homozygous ADH1B*1/1 genotype given by author, year, country, number of ADH1B*1/1 cases and controls, minor allele frequencies, HWE and odds ratios with confidence intervals (EA=European American, AA=African American, Chin.=Chinese, Cauc.=Caucasian, MA=Mexican American, NA=Native American).

We collected the data given for the ADH1B*1/1 genotypes and created a forest plot using the odds ratios and 95% confidence intervals we calculated:

Summary meta-analysis ADH1B*1/1 (1)

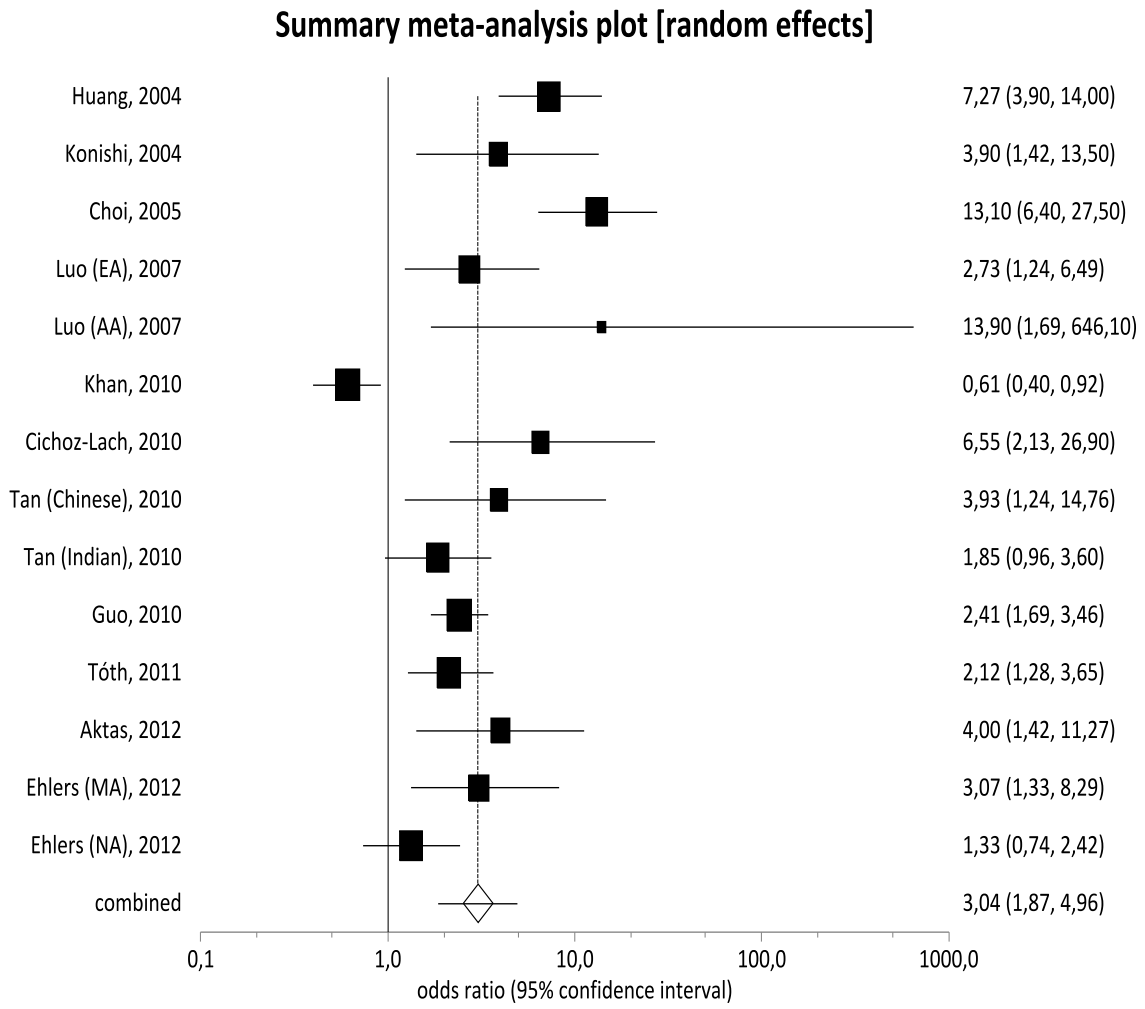


Figure 1.1
Association of the ADH1B*1/1 genotype with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (EA=European American, AA=African American, MA=Mexican American, NA=Native American).

Results of the ADH1B*1/1 (1) meta-analysis: The meta-analysis implied a significantly higher risk for alcohol dependence by being carrier of the ADH1B*1/1 gene variant (OR=3,04; 95% CI=1,87-4,96) throughout any ethnicity. The individual odds ratios ranged from 0,61 to 13,90. The Cochran Q test ($P=10^{-4}$) confirmed quite high between-study heterogeneity as well as the

Inconsistency-test (I^2=84,6%). This led to the application of a random effects model (DerSimonian-Laird).

The majority of the studies reported a higher risk for becoming alcohol dependent by being carrier of the ADH1B*1/1 genotype. This genotype leads to a switch from His (ADH1B*2 allele) to Arg as both genes contain adenines instead of guanines. That change in the alcohol dehydrogenase 1B isoenzyme is characterized by a slow metabolism, thus avoiding negative side effects from high acetaldehyde levels and enlarging the time of gaining benefit from the positive emotions after alcohol consumption. This justifies the bringing forward effect of this mutation in the development of alcoholism as nearly all of the studies state.

Discussion of the ADH1B*1/1 (1) meta-analysis: For example Guo et al. (2010), Tóth et al. (2011), Ehlers et al. (2012), Tan (Indian) et al. (2010) and Luo (EA) et al. (2007) deliver data that on the one hand stresses the importance of the homozygous ADH1B*1/1 genotype in the disease of alcoholism and on the other hand presents the significant results as the confidence intervals are quite small. This means the group size examined was high enough to produce reliable data as well as the standard deviation was quite small which also leads to a narrow confidence interval. In contrast to that some other studies revealed quite big confidence intervals: Luo (AA) et al. (2007), Konishi et al. (2004), Huang et al. (2004), Tan (Chinese) et al. (2010) and Cichoz-Lach et al. (2010). So these results have to be seen carefully as the size of the 95% CIs suggests that the true value can scatter immensely hence not giving significant results. Khan et al. (2010) did not support the other studies claiming a positive association between the ADH1B*1/1 genotype and alcohol dependence (OR=0,61, 95% CI:0,40-0,92) but without the control group sample meeting HWE (p=0,04) which stands for a deviation of the study's control sample from ideal population as no subjects were heterozygous carriers for the ADH1B alleles.

We created a funnel plot to detect publication bias from a possible deviation from the symmetry assumption as well as from Egger's regression test:

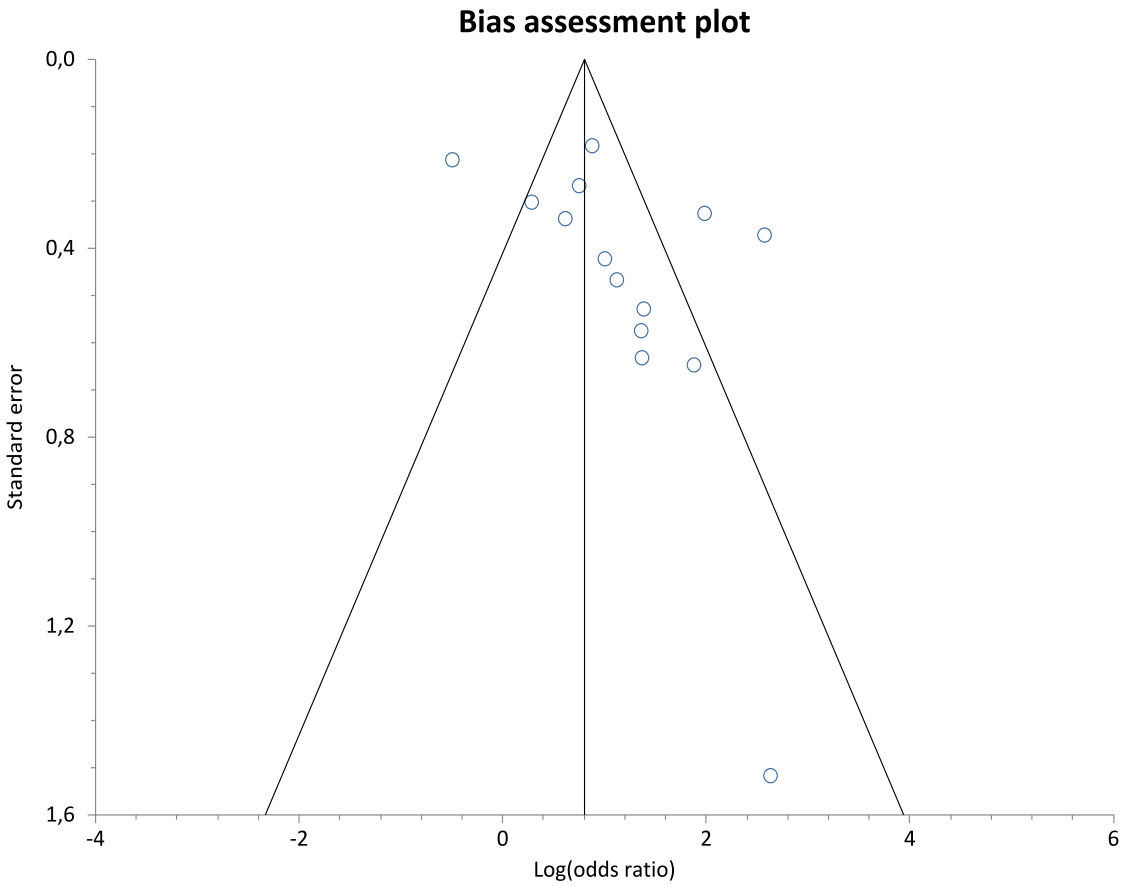


Figure 1.2
The funnel plot is showing log(OR) and standard error for the association of ADH1B*1/1 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,08.

Although the funnel plot doesn't indicate publication bias a few studies with a high number of participants showed some aberration from the mean value (Khan et al., 2010; Huang et al., 2004). Bigger studies should actually deliver results that are close to the mean value as the lines in the funnel plot suggest. The reason for the aberration of the study by Khan et al. (2010) probably lies in the problem with the control group sample not being compounded by an ideal population as they didn't meet HWE (p=0,04). The problem with the study by Huang et al. (2004) may lie in the fact that only 16 out of 281 controls were carriers for the ADH1B*1/1 genotype thus achieving a comparably very high rate of ADH1B*1/1 genotypes in the case sample and hence stating a higher effect of this mutation for the development of alcoholism as the other studies (OR=7,27).

As the study by Luo (AA) et al. (2007) dealt with only 48 control subjects of which 41 were carriers for the ADH1B*1/1 genotype and the rest consisted only of 7 heterozygous and no homozygous carrier for the ADH1B*2 allele, the data given by that study differs immensely from other studies. The 95% CI (1,69-646,1) emphasizes the lack of participants in the control sample. So does the study by Cichoz-Lach et al. (2010). They examined 204 cases of which 200 were homozygous for the ADH1B*1 allele and 172 controls of which 152 subjects were homozygous for that allele. The number of participants being not homozygous for the ADH1B*1 allele consequently was very small which leads to a large confidence interval. Other studies had the same problem dealing with little group sizes for certain allelic constellations after genotyping the participants. Therefore we excluded those studies with a 95% confidence interval bigger than 8 from another forest plot thus guaranteeing that only studies with high group sizes and little standard deviations indicated by the 95% confidence intervals have influence on the outcome of our meta-analysis. Furthermore the study by Khan et al. (2010) was excluded because the control group didn't meet Hardy-Weinberg-Equilibrium (p=0,04). By processing the second analysis, we attempted to gain more significant data:

Summary meta-analysis ADH1B*1/1 (2)

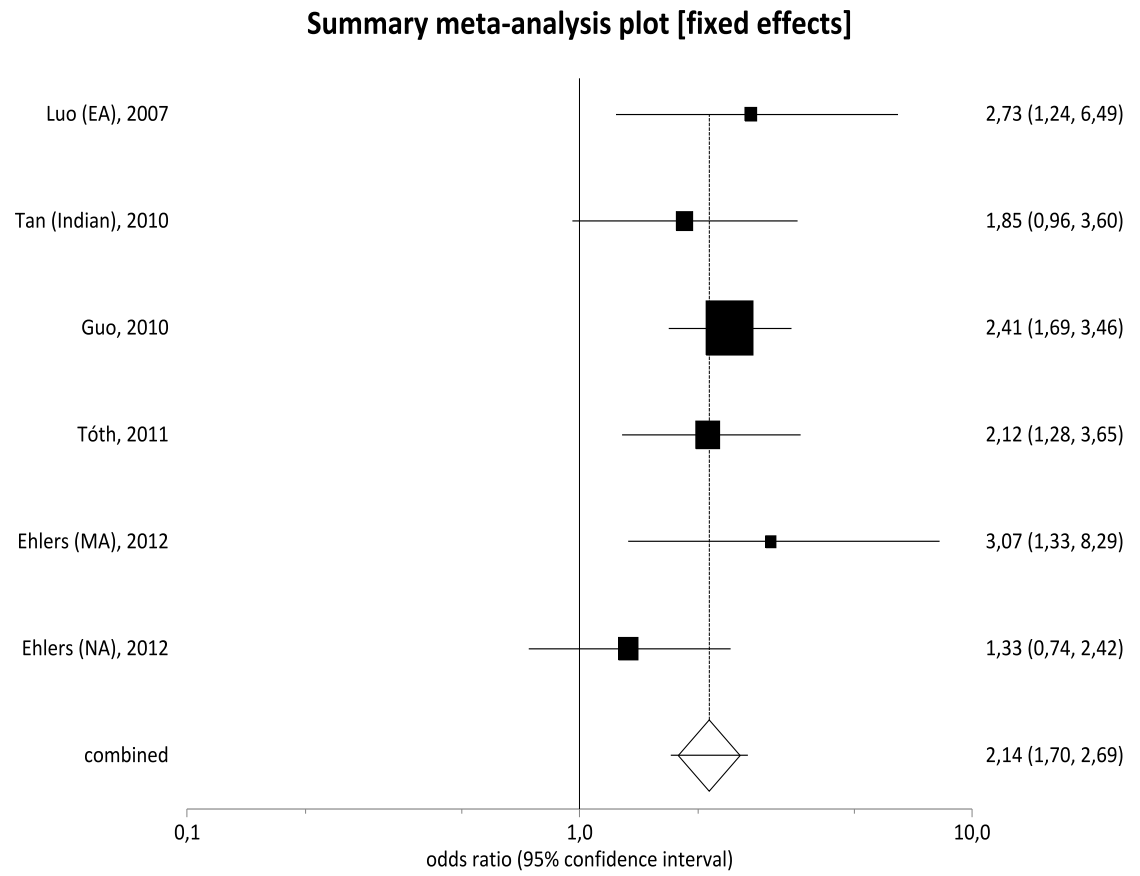


Figure 1.3
Association of the ADH1B*1/1 genotype with alcohol dependence after excluding several studies for the reasons mentioned above. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (EA=European American, MA=Mexican American, NA=Native American).

Results of the ADH1B*1/1 (2) meta-analysis: The meta-analysis implied a higher risk for alcohol dependence by being carrier of the ADH1B*1/1 gene variant (OR=2,14; 95% CI=1,70-2,69) throughout any ethnicity and delivered significant numbers. The individual odds ratios ranged from 1,33 to 3,07. The Cochran Q test (P=0,55) confirmed quite little between-study heterogeneity as well as the Inconsistency-test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel-Method). The 95% confidence interval of the combined effects (95%CI=1,70-2,69) became much smaller compared to the one of **Figure 1.1**. The likelihood of becoming alcohol dependent by being carrier of the ADH1B*1/1 genotype is reported to be twice as high compared to other genotypes of this isoenzyme.

Discussion of the ADH1B*1/1 (2) meta-analysis: The studies that were included in this second forest plot gave reliable data by dealing with high numbers of participants in both cases and controls as one can see in the sizes of the 95% CIs. As **Figure 1.1** shows, it may be not that easy to collect such high numbers of homozygous carriers for that allele as the confidence intervals suggest. However the bringing forward effect of this SNP in the ADH1B isoenzyme towards alcohol dependence is reported for any of the ethnicities examined (Native American, Mexican American, European American, Caucasian, Asian). So the meaning of this polymorphism for alcohol addiction seems to be massive in any of the analysed ethnicities.

In another funnel plot we were looking for publication bias:

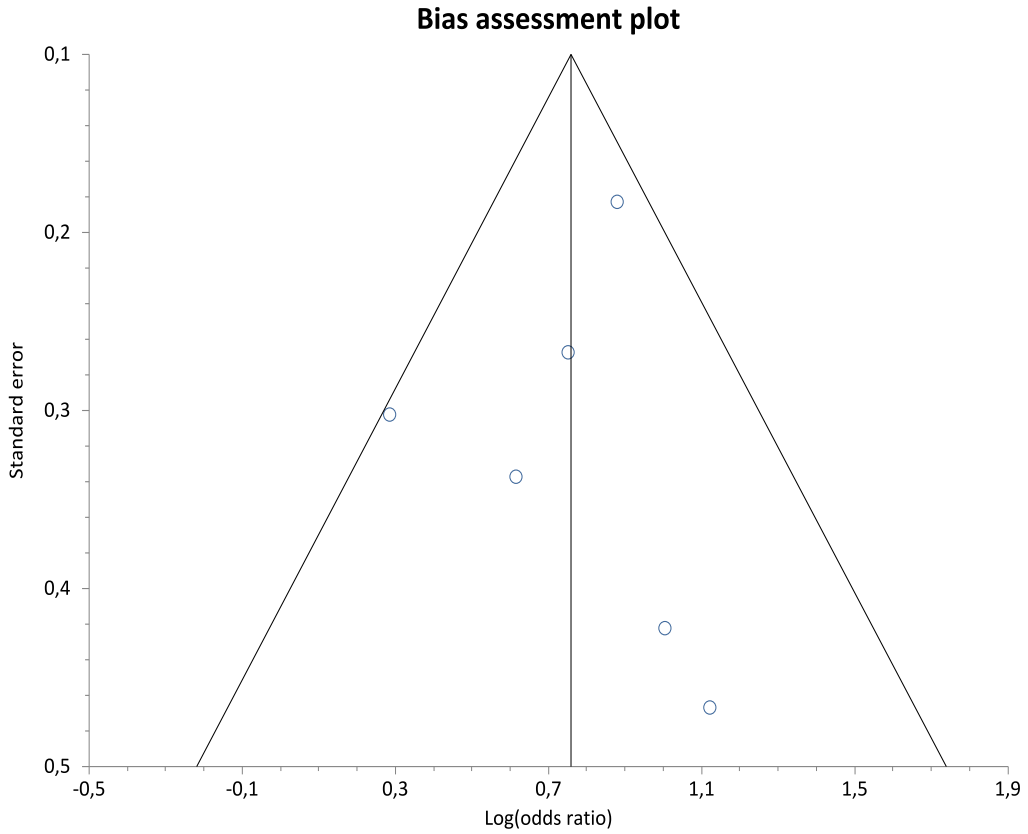


Figure 1.4
The funnel plot is showing log(OR) and standard error for the association of ADH1B*1/1 with alcohol dependence after including studies with smaller confidence intervals and controls groups meeting HWE only. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,97.

The ADH1B*2/2 genotype that leads to a faster isoenzyme (His/His) which is supposed reduce the risk of alcoholism has been analyzed by our meta-analysis too. While the ADH1B*1/1 genotype is associated with alcohol dependence, the ADH1B*2/2 should theoretically be more prevalent in the control samples of the studies we included.

We analyzed the same studies as we did for the ADH1B*1/1 genotype. Almost all the authors stated a protective effect of the ADH1B*2/2 genotype in the process of alcoholism (for example Guo et al., 2010; Huan et al., 2004; Choi et al., 2005). The problem with this SNP consisted in the very low frequency in several studies that made a statistical analysis impossible in some cases. This low amount of carriers of the ADH1B*2/2 genotype emphasizes the meaning of the ADH1B*2 allele as the single nucleotide polymorphism while the ADH1B*1 allele seems to represent the wildtype allele after calculating the minor allele frequencies in our analysis.

We collected the data given by the studies dealing with ADH1B*2/2 (guanine/guanine; 48His/48His) genotypes that met our inclusion criteria and summed the results up in **Table 1.2**:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Huang	2004	Taiwan	34	154	0,42	0,27	0,49	0,19 (0,12-0,29)
Konishi	2004	USA	1	2	0,02	0,05	0,06	0,63 (0,01-12,11)
Choi	2005	Korea	31	143	0,42	0,21	0,13	0,26 (0,15-0,44)
Luo, EA	2007	USA	2	0	0,03	0,06	0,35	-
Luo, AA	2007	USA	0	0	0,01	0,02	0,87	-
Khan	2010	India	57	54	0,1	0,08	0,04	1,3 (0,87-2,06)

Cichoz-Lach	2010	Poland	1	1	0,01	0,06	0,633	0,08 (0,01-66,5)
Tan, Chin.	2010	Malaysia	35	55	0,36	0,26	0,3	0,73 (0,38-1,36)
Tan, Indian	2010	Malaysia	13	12	0,34	0,43	0,26	0,81 (0,32-2,08)
Guo	2010	China	2	10	0,09	0,16	0,46	0,18 (0,02-0,85)
Tóth	2011	Hungary	0	2	0,04	0,09	0,15	-
Aktas	2012	Turkey	1	1	0,04	0,12	0,79	1,21 (0,074-19,7)
Ehlers, MA	2012	USA	0	1	0,03	0,08	0,71	-
Ehlers, NA	2012	USA	0	1	0,06	0,04	0,45	-
Bjerre-gaard, Inuit	2014	Denmark	0	0	0	0	<0,001	-
Bjerre-gaard, Cauc.	2014	Denmark	0	0	0	0	<0,001	-
Bjerre-gaard, Yupik	2014	Denmark	0	0	0	0	<0,001	-

[a]Number of cases being carrier for the ADH1B*2/2 genotype

[b]Number of controls being carrier for the ADH1B*2/2 genotype

[c]Minor allele frequencies for the populations' ADH1B*2/2 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the ADH1B*2/2 genotype and alcohol dependence

Table 1.2

Characteristics of the studies dealing with carriers for the homozygous ADH1B*2/2 genotype

given by author, year, country, number of ADH1B*2/2 cases and controls, minor allele frequencies, HWE and odds ratios with confidence intervals (EA=European American, AA=African American, Chin.=Chinese, Cauc.=Caucasian, MA=Mexican American, NA=Native American).

Then we took the numbers given by the studies to create a forest plot for the association between homozygous carriers of the potentially protective ADH1B*2 allele and the development of alcohol dependence:

Summary meta-analysis ADH1B*2/2 (1)

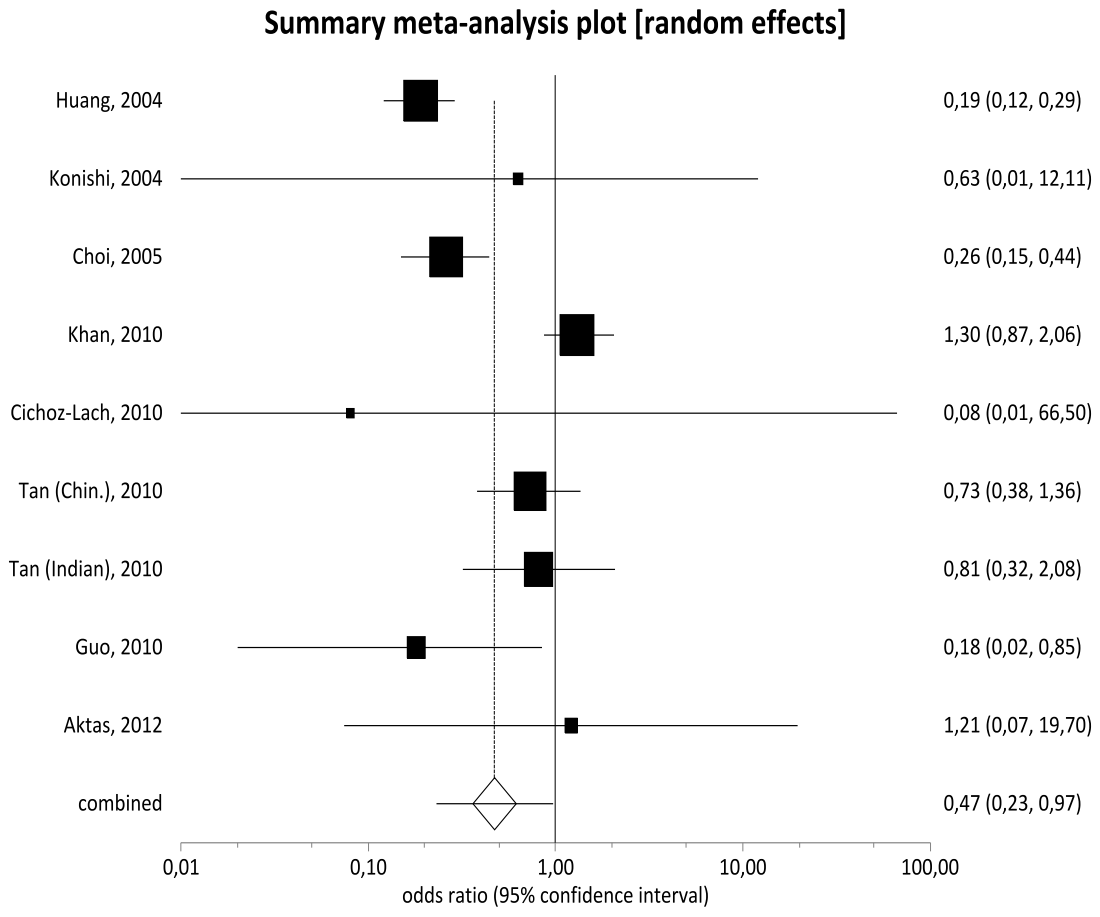


Figure 1.5
Association of the ADH1B*2/2 genotype with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (EA=European American, AA=African American, MA=Mexican American, NA=Native American).

Results of the ADH1B*2/2 (1) meta-analysis: The meta-analysis implied a significantly decreased risk for alcohol dependence by being carrier of the ADH1B*2/2 gene variant (OR=0,47; 95% CI=0,23-0,97). Thus the combined effect revealed significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,19 to 1,30. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=83,1%). This led to the application of a random effects model (DerSimonian-Laird).

Khan et al. (2010) and Aktas et al. (2012) reported a bringing forward effect of the ADH1B*2/2 genotype on the development of alcohol dependence without giving significant results. But the control population in the study of Khan et al. (2010) didn't meet HWE as we already know from the ADH1B*1/1 analysis. Furthermore the study of Aktas et al. (2012) only dealt with one case and one control being homozygous for the ADH1B*2 allele. In general we found lots of studies with a very little amount of ADH1B*2/2 carriers such as Cichoz-Lach et al. (2010), Guo et al. (2010) or the above mentioned study by Aktas et al. (2012). Other studies didn't even find one single subject with this genotype: Bjerregaard et al. (2014), Tóth et al. (2011), Luo et al. (2007) and Ehlers et al. (2012). What is more there exist much higher frequencies of this genotype in Asian populations than in other ethnic groups. All the studies that delivered results with a high number of ADH1B*2/2 subjects were examining Asian participants (Huang et al., 2004; Tan et al., 2010; Guo et al., 2010; Choi et al., 2005). Some Caucasian group samples didn't even contain a single carrier for this homozygous genotype (Bjerregaard et al., 2014; Tóth et al., 2011). The same situation occurred in American group samples (Luo et al., 2007).

Discussion of the ADH1B*2/2 (1) meta-analysis: As several studies deal with a very little amount of participants with the ADH1B*2/2 genotype the results can be questioned as the large confidence intervals already suggest. Hence we consider this allele to be the mutation while the allele leading to the amino acid Arg at this position (ADH1B*1) represents the wildtype. The majority of the studies however reported the expected effect of that allele, named a decreased likelihood of becoming alcohol dependent by being homozygous carrier of the ADH1B*2 allele which encodes a fast metabolizing ADH1B isoenzyme. The quite high frequency of the ADH1B*2 allele in Asian populations explains a certain protection from

alcoholism of this ethinc group in comparison with people of other ethnic backgrounds (Huang et al., 2004).

We created a funnel plot to detect publication bias in the studies included in our meta-analysis:

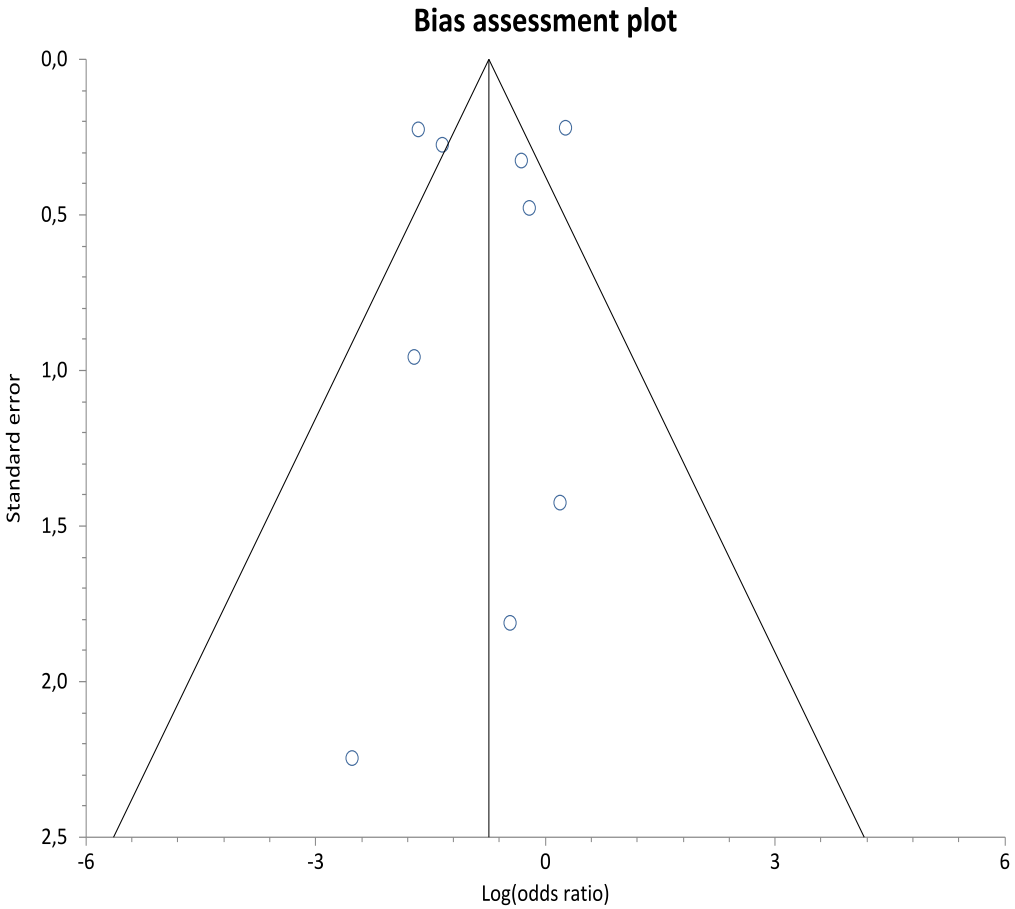


Figure 1.6
The funnel plot is showing log(OR) and standard error for the association of ADH1B*2/2 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,94.

As a quality assessment we created a second forest plot just including studies that dealt with control samples that met HWE and samples of participants that could collect enough homozygous carriers of the ADH1B*2 allele which was expressed in the confidence intervals that shouldn't be larger than 8 in our second forest plot:

Summary meta-analysis ADH1B*2/2 (2)

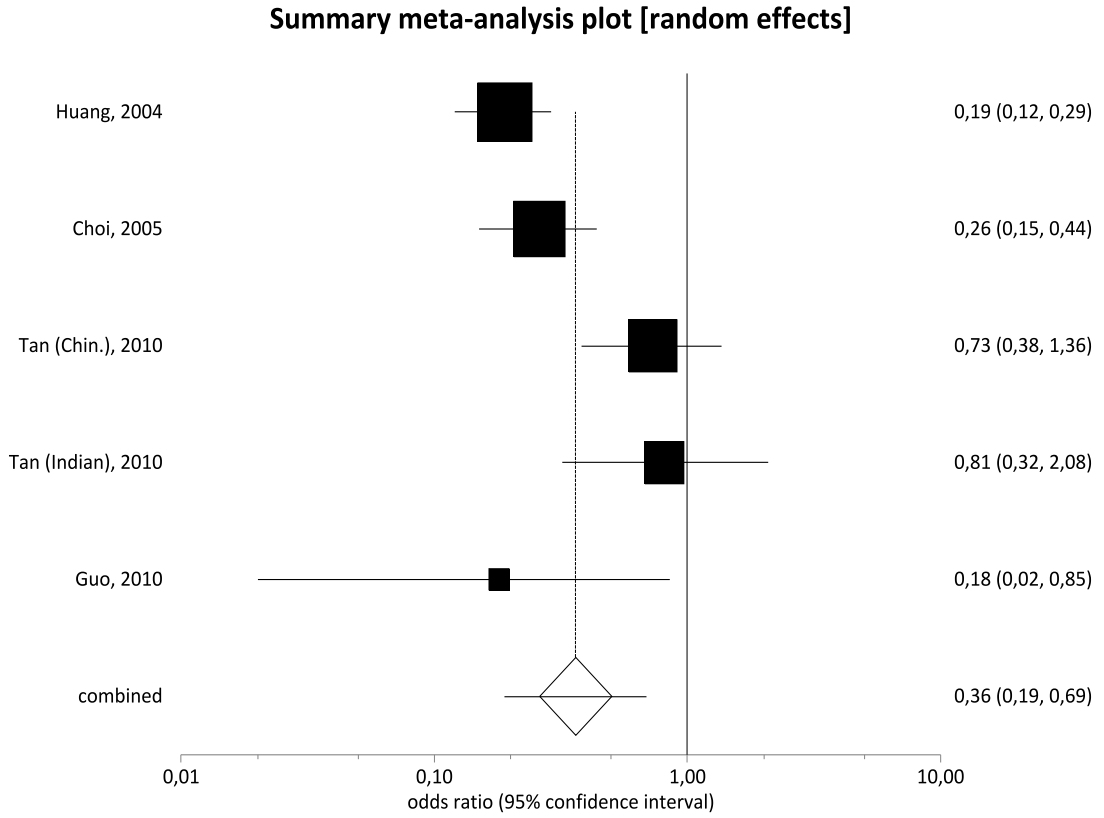


Figure 1.7
Association of the ADH1B*2/2 genotype with alcohol dependence after excluding several studies for the reasons of not meeting HWE in the control group either or of not giving significant numbers of a sufficiently big examination sample by taking into consideration the range of the study's confidence interval. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the ADH1B*2/2 (2) meta-analysis: The meta-analysis implied a significantly decreased risk for alcohol dependence by being carrier of the ADH1B*2/2 gene variant (OR=0,36; 95% CI=0,19-0,69). The individual odds ratios ranged from 0,19 to 0,81. The Cochran Q test (P=0,003) confirmed quite high between-study heterogeneity as well as the Inconsistency-test (I^2=75,7%). This led to the application of a random effects model (DerSimonian-Laird).

Discussion of the ADH1B*2/2 (2) meta-analysis: The studies we could include in the second meta-analysis for the ADH1B*2/2 genotype were all dealing with Asian participants. Homozygosity of the ADH1B*2 allele only seemed to be highly prevalent in Asian populations, while other studies that analyzed other ethnicities couldn't report any association of the mutation with alcoholism because the number of homozygous carriers of the protective allele was too small to achieve significant data (Ehlers et al., 2012; Aktas et al., 2012; Cichoz-Lach et al., 2010; Tóth et al., 2011). Both, cases and controls, were missing genetic information for the fast-metabolizing isoenzyme of the alcohol dehydrogenase. So, Asian subjects have smaller tolerance to ethanol consumption by disintegrating the substrate more quickly and hence suffering from negative effects caused by acetaldehyde for a longer time and in a higher intensity than other ethnic groups. This fact might explain why Asians are supposed to bear less alcohol consumption than Europeans or North American subject in popular belief. After all there seems to exist a protection from alcoholism in the Asian ethnicity in form of a SNP in the ADH1B gene (adenine→guanine/Arg48His) that does not reach the same significance in other ethnic groups because of the low prevalence.

We were using a funnel plot to detect publication bias in the studies we included in the second meta-analysis about the ADH1B*2/2 genotype and its meaning for the development of alcohol dependence:

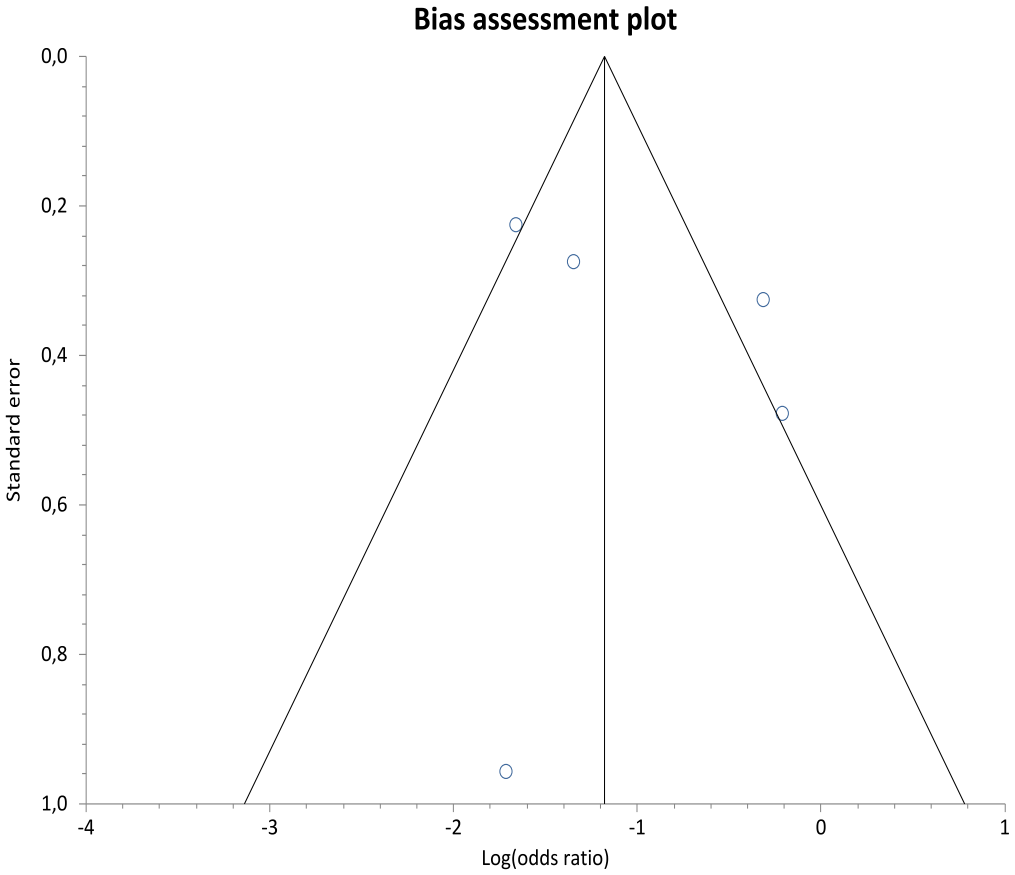


Figure 1.8
The funnel plot is showing log(OR) and standard error for the association of ADH1B*2/2 with alcohol dependence after including studies with confidence intervals smaller than 8 and controls groups meeting HWE only. Thus we were only referring to Asian individuals because they were the only ethnic group with a sufficient amount of homozygous carriers of the ADH1B*2 allele to be able to calculate significant data for. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,52.

The alleles of the ADH1C gene sequence have also been taken into consideration for being responsible for an increased risk of becoming an alcoholic. Together with the above mentioned mutations of the ADH1B alleles, they looked for an association in a population of 99 alcohol dependent subjects and 225 control subjects from Seoul (Choi et al., 2005). Two variants of the genetic area of the ADH1C genetic sequence, that encode for a slow isoenzyme, were linked with a severely enlarged likeliness for developing an alcohol

dependence. One of them was the ADH1C Ile350Val polymorphism located on rs689. The ADH1C*1 allele (adenine) acts protectively against alcohol dependence as it encodes for a faster isoenzyme which increases the amount of acetaldehyde leading to negative feelings after drinking. On the other hand the ADH1C*2 allele (guanine) encodes for the isoenzyme with a slower metabolism. Hence the positive feelings after ethanol consumption prevail and regular drinking becomes more likely (Li et al., 2012). In the study by Choi et al. (2005) there were no control subjects being carrier for the ADH1C*2/2 high-risk genotype. Also in other examinations especially the ADH1C Ile350Val (ADH1C*1/ADH1C*2) alleles were thought to have an association with alcohlism. We examined the homozygous carriers for these two alleles and examined if they differed in their individual drinking behaviour in a significant manner as some studies reported (Cichoz-Lach et al., 2010; Bjerregaard et al., 2014).

Another study by Ehlers et al. (2012) didn't describe that effect of the polymorphism in the ADH1C gene on alcohol dependence. They could reveal results from an examination of 924 Native Americans and 522 Mexican Americans who were giving evidence for an association between alcohol dependence and ADH1B and ALDH2 polymorphisms but they did not draw a linkage between ADH1C polymorphisms and that disorder (high risk genotype ADH1C*2/2: OR(Native Americans)=1,13; OR(Mexican Americans)=0,92).

Aktas et al. (2012) did not just examine the meaning of the ADH1B polymorphisms on alcoholism in a Turkish population of 75 cases and 100 controls but also the importance of mutations of another isoenzyme of the ADH, the ADH1C, in the same population. They failed to state an impact of the alleles on the risk of alcoholism. ADH1C*1/1 (Ile/Ile) carriers especially were not protected from alcohol dependence in a significant way in that population sample (OR=0,52; 95% CI: 0,18-1,56). ADH1C*2/2 (Val/Val) participants should theoretically have a higher risk of becoming alcohol dependent by disintegrating ethanol slowly. In this study though, there was no significantly increased predisposition to the addiction by being homozygous carrier of the genetic polymorphism (OR=1,25; 95% CI: 0,69-2,29).

Focusing on ADH1B polymorphisms (Arg48His)and ADH1C polymorphisms (Ile350Val), 241 cases with long-term alcohol abuse and chronic liver disease and 666 randomly selected controls without liver diseases were analyzed and genotyped (Tóth et al., 2011). They registered especially the meaning of the two ADH1B polymorphisms for being delicately

towards alcoholism. In particular the homozygous form of the ADH1B*1 allele was associated with alcohol dependence. The two alleles of the ADH1C leading to the amino acid switch Ile350Val did not alter the risk of regular drinking significantly in this Caucasian population.

A study with a very large number of participants took part in Denmark (Bjerregaard et al., 2014). The participants were divided into three different subgroups dependent on their ethnic background. The first subgroup consisted of Inuit individuals from Greenland. The examiners genotyped 4162 Inuit participants and divided them into drinkers and non-drinkers with the help of a self-administered questionnaire about their individual drinking pattern. The questionnaire was a modified CAGE questionnaire (Zierau et al., 2005). They observed an association between the ADH1C*2/2 (guanine/guanine) genotype and regular drinking (OR=1,38; 95% CI: 1,12-1,71). Moreover they also reported a protective effect of the fast metabolizing isoenzyme of ADH1C*1/1 genotype (adenine/adenine). Thus this subgroup confirms the association of the polymorphisms of the ADH1C with alcoholism. The second subgroup in the study by Bjerregaard et al. (2014) was assembled by Caucasians living in Denmark. They all together counted 9080 cases and 3631 controls. They were genotyped and asked about their drinking behaviour in earlier studies by Husemoen et al. (2008). So the examined population was very big thus being able to deliver significant and reliable results. The outcome of their analysis was controversy to the one of several other studies though not following the biochemical idea of a slow ADH increasing the risk of alcohol dependence. The homozygous ADH1C*2/2 carriers were negatively associated with alcoholism (OR=0,82; 95% CI: 0,75-0,91), while the homozygous carriers of the ADH1C*1 allele, which encodes a fast disintegrating isoenzyme that should actually act protectively, had an increased risk for alcoholism (OR=1,26; 95% CI:1,16-1,37). So the results for this population deliver a completely different association of the genotypes with alcoholism than other studies and even than other examined groups in the same study do. The Yupik Escimo population from Alaska consisted of 69 cases and was referred to 3631 non-alcoholic controls too. The direction of association followed the one of the Inuit sample in case of the high-risk genotype ADH1C*2/2 (OR=1,95; 95% CI: 1,17-1,95). The ADH1C*1/1 genotype didn't show any alteration in risk of becoming alcohol dependent as the OR lay at 0,99. The study by Bjerregaard et al. (2014) also considered ADH1B and ALDH2 genotypes to be associated with alcohol dependence but there were no subjects found for the homozygous protective ADH1B*2 allele as well as for the ALDH2*1/1 genotype. This lack of the homozygous carriers

for these alleles is very surprising because the population sample was very big, in case of the Caucasian population even 9080 cases and 3631 control subjects. Nevertheless although the group sizes were big enough to fulfil our inclusion criteria, there was no statistical analysis possible for two of the three examined single nucleotide polymorphisms.

A Turkish sample of 90 cases and 100 controls was analyzed for their ADH1C genotypes in a study by Kortunay et al. (2012). Although the authors alluded that there existed some differences in the educational status between case and control subjects and the fact that the mean age of the case sample lay at about 43 years while the mean age of the controls was only 35 years, there was a connection between the ADH1C*2 allele and an increased risk for becoming alcohol dependent in this study. The homozygous genotype of the ADH1C*2 allele wasn't even present among the control subjects during there were 6 cases being homozygous carriers for this allele. This may stress the importance of the ADH1C*2/2 genotype for the development of alcoholism but made it impossible to include the data of this study in our meta-analysis either. On the other hand the ADH1C*1/1 genotype was significantly associated with a protective effect towards alcoholism (OR=0,2; 95% CI: 0,1-0,4).

We had to exclude studies from the meta-analysis because they were not showing the genotypes of the examined subjects: Kuo et al. (2008), Kim et al. (2008), Gizer (2011), Mulligan (2003), Treutlein et al. (2009). Other studies were not dealing with control samples: Abulseoud et al., (2013), Homann et al. (2006), Linneberg et al., (2010), Tolstrup et al. (2008). Those studies had to be excluded as well.

We gleaned the data given by studies dealing with ADH1C*2/2 (guanine/guanine; 350Val/350Val) genotypes that met our inclusion criteria and summed it up in **Table 1.3**:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Konishi	2004	USA	28	37	0,39	0,34	0,02	0,5 (0,28-0,9)
Choi	2005	Korea	5	0	0,15	0,05	0,41	-

Luo, EA	2007	USA	59	48	0,42	0,40	0,34	2,32 (1,46-3,7)
Luo, AA	2007	USA	1	1	0,19	0,13	0,78	0,5 (0,01-40,1)
Khan	2010	India	163	259	0,28	0,34	<0,001	0,53 (0,38-0,72)
Cichoz-Lach	2010	Poland	31	64	0,37	0,4	0,27	0,3 (0,18-0,51)
Tóth	2011	Hungary	36	94	0,39	0,39	0,33	1,07 (0,68-1,64)
Aktas	2012	Turkey	41	49	0,26	0,32	0,34	1,25 (0,69-2,29)
Ehlers, MA	2012	USA	14	37	0,32	0,31	0,09	0,92 (0,44-1,83)
Ehlers, NA	2012	USA	73	54	0,38	0,35	0,02	1,13 (0,76-1,7)
Kortu-nay	2012	Turkey	6	0	0,32	0,13	0,19	-
Bjerre-gaard (Yupik)	2014	Denmark	27	763	0,45	0,46	0,7	1,95 (1,17-1,95)
Bjerre-gaard (Inuit)	2014	Denmark	143	763	0,49	0,46	0,7	1,38 (1,12-1,71)
Bjerre-gaard (Cauc.)	2014	Denmark	1634	763	0,42	0,46	0,7	0,82 (0,75-0,91)

[a]Number of cases being carrier for the ADH1C*2/2 genotype

[b]Number of controls being carrier for the ADH1C*2/2 genotype

[c]Minor allele frequencies for the populations' ADH1C*2/2 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the ADH1C*2/2 genotype and alcohol dependence

Table 1.3
Characteristics of the studies dealing with carriers for the homozygous ADH1C*2/2 genotype given by author, year, country, number of ADH1C*2/2 cases and controls, minor allele frequencies, HWE and odds ratios with 95% confidence intervals (EA=European American, AA=African American, Cauc.=Caucasian, MA=Mexican American, NA=Native American).

As we consider by studying **Table 1.3** and comparing the numbers with the total amount of cases and controls given by **Table 1.0**, we can regard the ADH1C*2 allele as the SNP while the ADH1C*1 allele is supposed to be the wildtype as the populations in the majority of the studies, and especially the control samples, deal with higher frequencies of this allele. The ADH1C*2 allele is less frequent in most of the studies (Luo et al., 2007; Kortunay et al., 2007; Konishi et al., 2004; Ehlers et al., 2012).

The results of all the studies dealing with the ADH1C*2/2 genotype and its meaning for the development of alcoholism are summed up and presented in the Forest Plot below:

Summary meta-analysis ADH1C*2/2 (1)

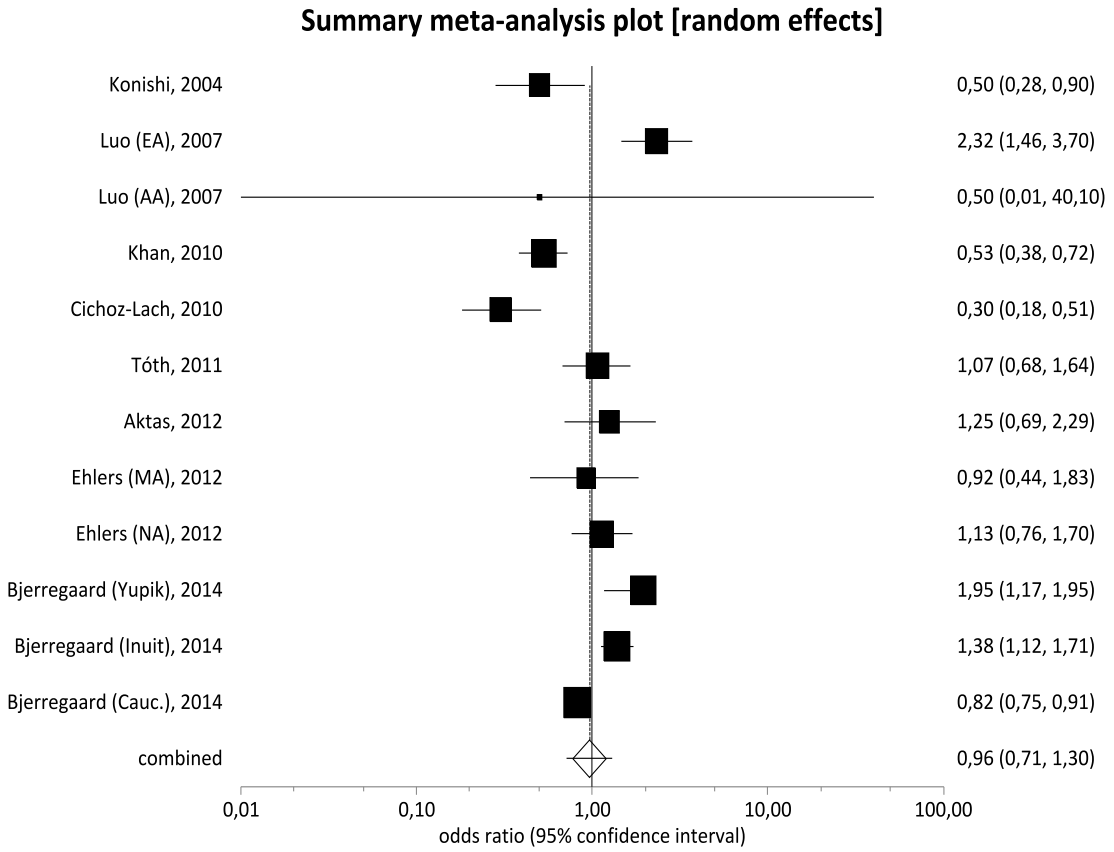


Figure 1.9
Association of the ADH1C*2/2 genotype with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (EA=European American, AA=African American, MA=Mexican American, NA=Native American, Cauc.=Caucasian).

Results of the ADH1C*2/2 (1) meta-analysis: The meta-analysis implied no significantly decreased or increased risk for alcohol dependence by being carrier of the ADH1C*2/2 gene variant (OR=0,96; 95% CI=0,71-1,30). Thus the combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,30 to 2,32. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=89,4%). This led to the application of a random effects model (DerSimonian-Laird).

Several studies suggest that there is no association between the SNP and the likeliness of becoming an alcoholic, delivering an OR at about 1 (Ehlers et al., 2012; Tóth et al., 2011; Bjerregaard et al. (Cauc.), 2014; Aktas et al., 2012). After all there are more studies reporting no effect or at least a weak association between the ADH1C*2/2 genotype and alcoholism. The SNP leading to the amino acid Val should actually reduce the catalytic activity of the ADH1C and thus increase the risk for alcoholism by benefiting longer times from the positive feeling after alcohol consumption and lowering the time acetaldehyde is circulating and causing aversive somatic and psychological feelings (Li et al., 2012). Nevertheless four studies report a negative association between the homozygous ADH1C*2/2 genotype and alcoholism such as Cichoz-Lach et al., 2010; Luo (AA) et al., 2007; Khan et al., 2010; Konishi et al., 2004. First of them stated even a younger age of the ADH1C*1/1 genotype when first regular alcohol consumption becomes registered (Cichoz-Lach et al., 2010). However Luo (AA) et al. (2007) just collected one carrier of the ADH1C*2/2 genotype in both case and control group.

Discussion of the ADH1C*2/2 (1) meta-analysis: The control group by the study of Khan et al. (2010) didn't meet HWE ($p<0,001$). Hence these results have to be questioned which is why we excluded them from a second meta-analysis, just including studies that met HWE in the control sample plus reported little standard deviations as well as high numbers of participants. The last two properties were evaluated by the size of the 95% CI. We only included studies with a 95% confidence interval smaller than 8.

Another fact becomes obvious. During our meta-analysis about the ADH1B polymorphisms included lots of studies dealing with Asian participants (for example Tan et al., 2010; Huang et al., 2004; Choi et al., 2005), there was only one study with Asian participants included in our meta-analysis of the ADH1C polymorphisms (Khan et al., 2010). This study didn't even meet HWE in the control group thus probably delivering results that are not just dependent on genetic variation. The study by Choi et al. (2005) could not find any carrier of the ADH1C*2/2 genotype in the control group, the minor allele frequency was only 5% for the control sample. Altogether there is only a very little amount of studies dealing with Asian participants and the issue if the ADH1C polymorphisms alter their risk of becoming alcohol dependent. This might be caused by the low availability of this potential high-risk genotype which would support the findings of the ADH1B*2/2 polymorphisms, representing Asian populations as genetically better protected individuals from alcoholism than other ethnic

groups or the fact that this SNP was just not considered to play a role in the progress of alcohol dependence. The Inuit population in the study by Bjerregaard et al. (2014) represents an exception. They are also originally of Asian ancestry but emigrated long time ago from Asia to Greenland. They have a higher prevalence of alcohol dependence in their general population than other Asian populations (Bjerregaard et al., 2014). One reason might consist of the higher amount of ADH1C*2/2 carriers. The other ethnicities mostly show no association between the ADH1C*2/2 genotype and alcoholism or a slightly positive (Bjerregaard et al., 2014; Luo (EA) et al., 2007) or a slightly negative effect (Luo (AA) et al., 2007; Cichoz-Lach et al., 2014). There is no clear direction of association recognisable.

We checked if publication bias exists by using a funnel plot and Egger's regression test:

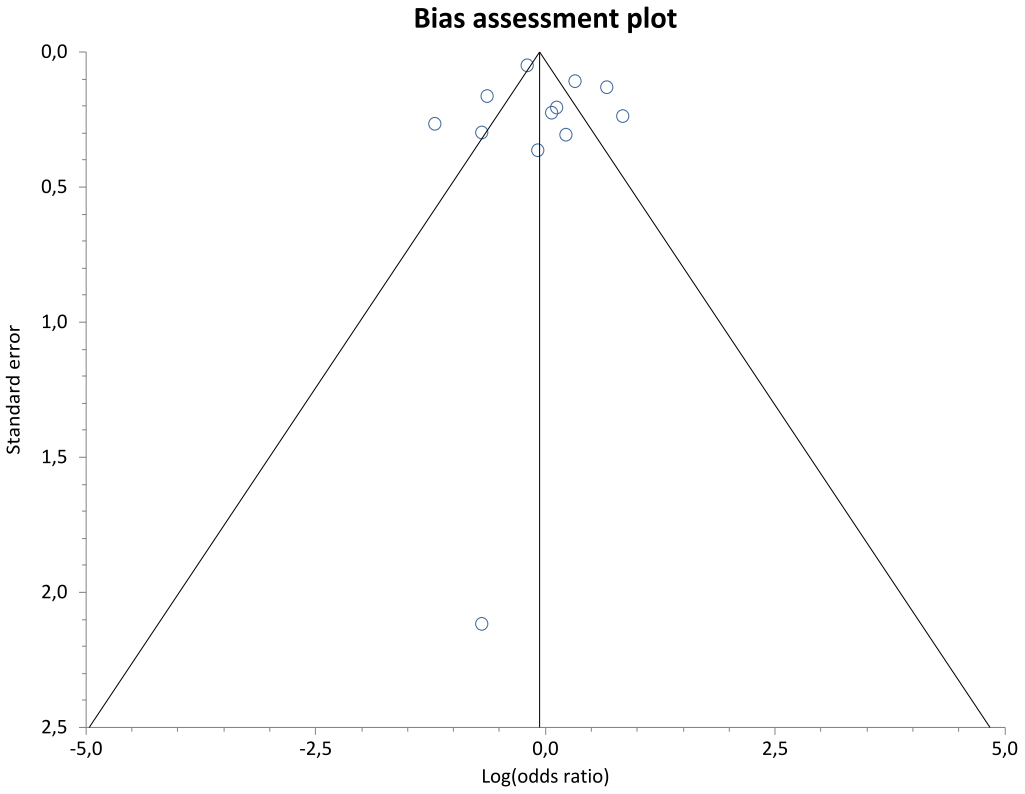


Figure 1.10
The funnel plot is showing log(OR) and standard error for the association of ADH1C*2/2 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,76.

We analyzed the studies with control groups meeting the HWE and confidence intervals smaller than 8 to make sure that the number of examined subjects was high enough to achieve significant results by the 95% CI not including the one. Therefore we created another forest plot:

Summary meta-analysis ADH1C*2/2 (2)

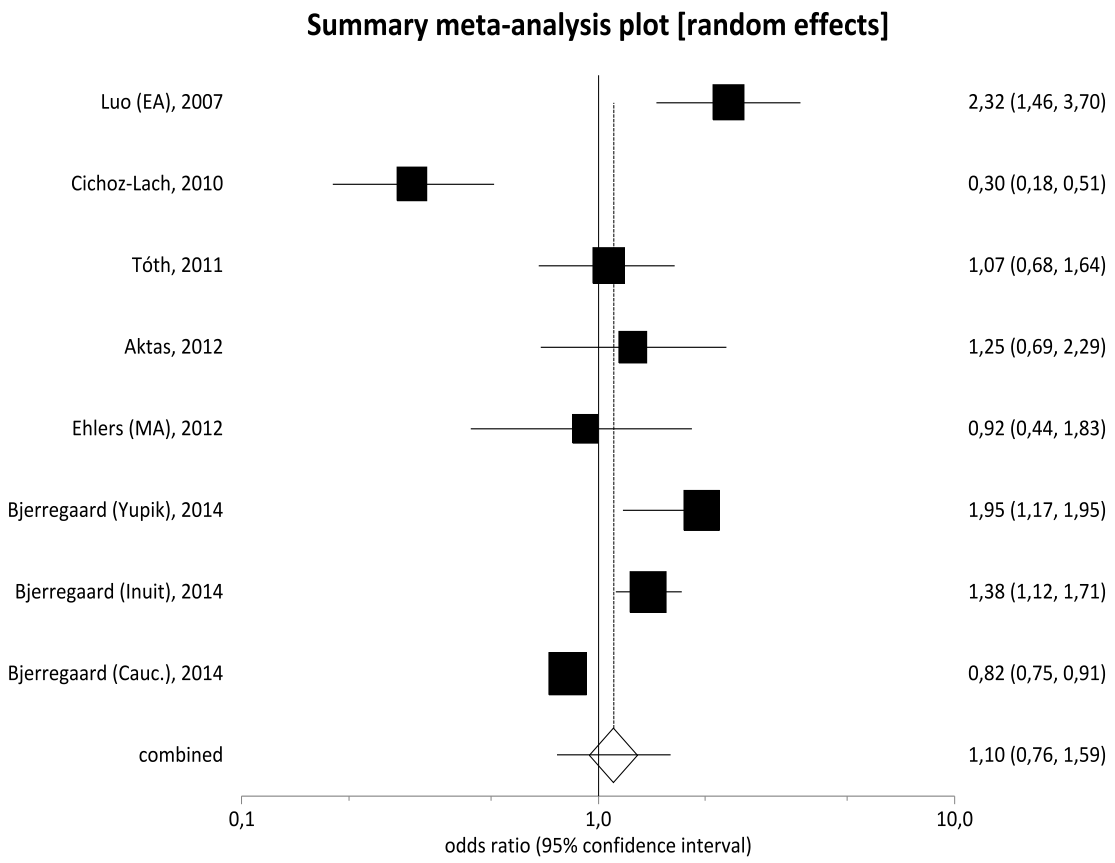


Figure 1.11
Association of the ADH1C*2/2 genotype with alcohol dependence after excluding several studies because they didn't meet HWE in the control group either or they didn't give significant numbers due to the very large range of the study's confidence interval. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the ADH1C*2/2 (2) meta-analysis: The meta-analysis implied no significantly decreased or increased risk for alcohol dependence by being carrier of the ADH1C*2/2 gene variant (OR=1,10; 95% CI=0,76-1,59). Thus the combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,30 to 2,32. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=91,8%). This led to the application of a random effects model (DerSimonian-Laird).

Even after excluding some studies for the above mentioned reasons, there is no association between the ADH1C*2/2 and alcoholism visible. So the studies that deliver significant associations between the SNP and alcohol addiction have to be supported by other studies in the future to establish the ADH1C*2/2 genotype as a high-risk genotype for alcohol dependence.

Discussion of the ADH1C*2/2 (2) meta-analysis: Nowadays there doesn't exist a sufficient number of results that report any association although the biochemical activity of the isoenzyme is reduced. It seems that this SNP is not altering the overall alcohol metabolism in a way the ADH1B*1/1 does, namely having a significant amount of ethanol circulating in the blood for a longer time and reducing the time acetaldehyde is causing its aversive symptoms. Altogether the ADH1C*2/2 does not alter the risk of becoming alcohol dependent, it seems that the clinical effect of the altered isoenzyme activity is not recognisable as it doesn't alter the individual drinking behaviour in a significant way.

We created a funnel plot to detect publication bias in the studies included in our second forest plot for the ADH1C*2/2 genotype:

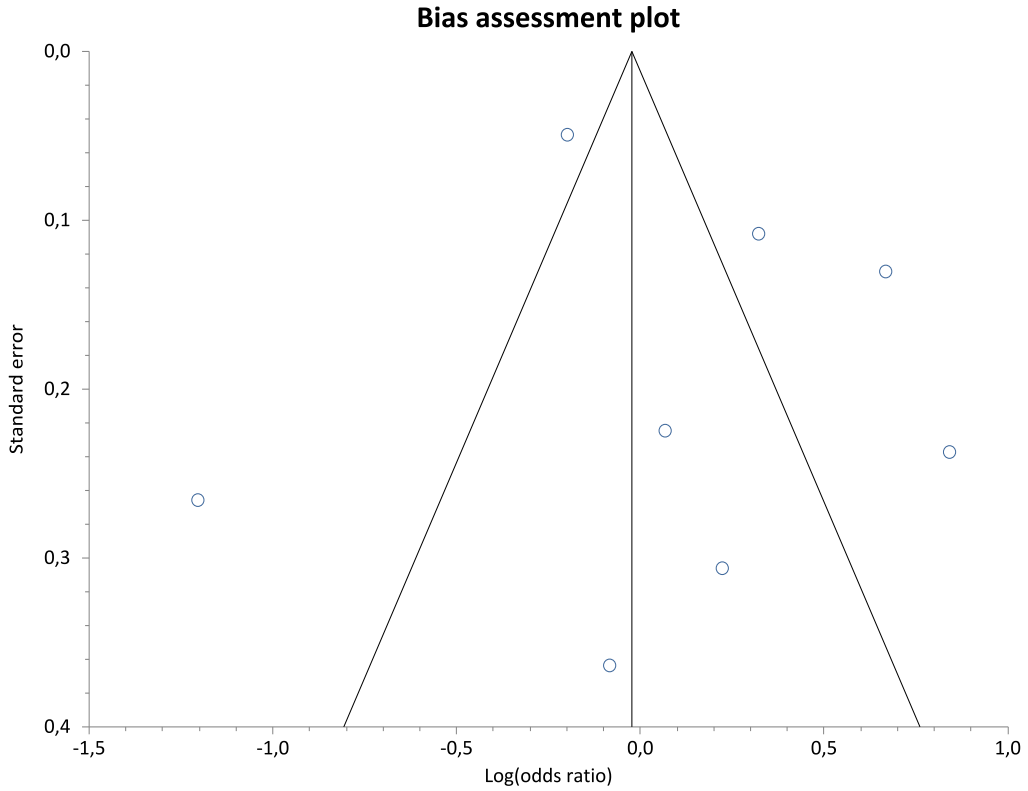


Figure 1.12
The funnel plot is showing log(OR) and standard error for the association of ADH1C*2/2 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,44.

As the allele encoding for an isoenzyme with fast pharmacokinetics, the ADH1C*1 polymorphism could represent a protective SNP for the likeliness of becoming an alcoholic. How big the influence of this allele is on the whole ethanol metabolism in the human organism was subject of several studies by examining the meaning of the ADH1C*1 allele for the development of alcoholism. We regarded the meaning of the ADH1C*1/1 genotype for alcohol dependence. Some studies dealt with the importance of that allele in different ethnic populations. For example Kortunay et al. (2012) analyzed a group of 90 alcohol dependent subjects between 20 and 78 years old who were consuming at least 80 g ethanol a day and compared the results with 100 controls. Moreover there was a study with Polish male

alcoholics processed to evaluate the influence of this polymorphism. They examined 204 male patients and 172 healthy subjects between 18 and 70 years old (Cichoz-Lach et al., 2010).

Bjerregaard et al. (2014) examined three different populations for the association between the ADH1C*1/1 genotype with alcohol dependence. For every ethnic group, they gained different results, once stating a negative, once a positive and in one case no association between the genotype and alcoholism.

In a Mexican American and a Native American population sample the ADH1C*1/1 genotype showed no negative association with alcohol dependence (Ehlers et al., 2012). Another study with Mexican Americans reported a protective effect of this genotype towards the development of alcoholism (Konishi et al., 2004). The majority of the studies found less carriers of the ADH1C*2 allele than carriers of the ADH1C*1 allele. Hence we considered the ADH1C*2 allele (guanine) to be the SNP while the amino acid Ile could be found in the major part of the populations. The efficiency of the isoenzyme of the ADH1C encoded by allele 1 is supposed to be higher than the one encoded by allele 2 (Li et al., 2012). So the group of people being carrier for the ADH1C*1/1 genotype should theoretically be protected from alcoholism in comparison to the ones not being homozygous carrier for this allele. How big the influence of this polymorphism is on the altogether risk for becoming alcohol dependent will be investigated in **Figure 1.13**. The issue is, if its influence is similar to the one of the ADH1B homozygous polymorphisms, which was significant for both homozygous gene variations, or if it is more like the meaning of the ADH1C*2/2 genotype that did not show significant change in the clinical risk of becoming alcohol dependent in comparison with other ADH1C genotypes.

We collected the data given by the studies included dealing with ADH1C*1/1 (adenine/adenine; 350Ile/350Ile) genotypes and alcohol dependence and summed the numbers up in **Table 1.4**:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Konishi	2004	USA	73	119	0,39	0,34	0,02	0,64 (0,43-0,95)
Choi	2005	Korea	76	192	0,15	0,05	0,41	0,56 (0,3-1,08)
Luo, AA	2007	USA	57	35	0,19	0,13	0,78	0,53 (0,21-1,24)
Luo, EA	2007	USA	108	105	0,42	0,4	0,34	2,45 (1,64-3,66)
Khan	2010	India	152	136	0,28	0,34	<0,001	1,6 (1,23-2,3)
Cichoz-Lach	2010	Poland	85	32	0,37	0,4	0,27	3,12 (1,9-5,19)
Tóth	2011	Hungary	90	244	0,39	0,39	0,33	1,03 (0,75-1,41)
Aktas	2012	Turkey	5	12	0,26	0,32	0,34	0,52 (0,18-1,56)
Ehlers, MA	2012	USA	58	153	0,32	0,31	0,09	0,88 (0,57-1,36)
Ehlers, NA	2012	USA	175	160	0,38	0,35	0,02	0,83 (0,62-1,11)
Kortu-nay	2012	Turkey	38	77	0,32	0,13	0,19	0,2 (0,1-0,4)
Bjerre-gaard (Yupik)	2014	Denmark	20	1053	0,45	0,46	0,7	0,99 (0,56-1,72)
Bjerre-gaard (Inuit)	2014	Denmark	127	1053	0,49	0,46	0,7	0,77 (0,62-0,95)

Bjerre-gaard (Cauc.)	2014	Denmark	3087	1053	0,42	0,46	0,7	1,26 (1,16-1,37)

[a]Number of cases being carrier for the ADH1C*1/1 genotype

[b]Number of controls being carrier for the ADH1C*1/1 genotype

[c]Minor allele frequencies for the populations' ADH1C*1/1 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the ADH1C*1/1 genotype and alcohol dependence

Table 1.4
Characteristics of the studies dealing with carriers for the ADH1C*1/1 genotype given by author, year, country, number of ADH1C*1/1 cases and controls, minor allele frequencies, HWE and odds ratios with confidence intervals (EA=European American, AA=African American, Cauc.=Caucasian, MA=Mexican American, NA=Native American).

The data advises that the ADH1C*1/1 genotype can be found more frequently than the ADH1C*2/2 genotype by regarding the allele frequencies. Therefore we could calculate useful confidence intervals for the respective population. In every study there existed enough participants in both cases and controls to give reasonable numbers. This is not self-evident when investigating the meaning of homozygous alleles as we have already noticed by analyzing the numbers for other polymorphisms. In this case though there was enough data delivering useful results as the 95% confidence intervals are all quite narrow. This is why we considered the ADH1C*1 allele to be the wildtype.

Data given by the studies of Duranceaux et al. (2006), Hines et al. (2005) and Lee et al. (2004) had to be excluded because they delivered the results in an idiosyncratic way that could not be used for our meta-analysis requiring case-control studies to be able to calculate ratios.

The results of all the studies dealing the ADH1C*1/1 genotype and its meaning for the development of alcoholism are summed up and presented in the Forest Plot below:

Summary meta-analysis ADH1C*1/1 (1)

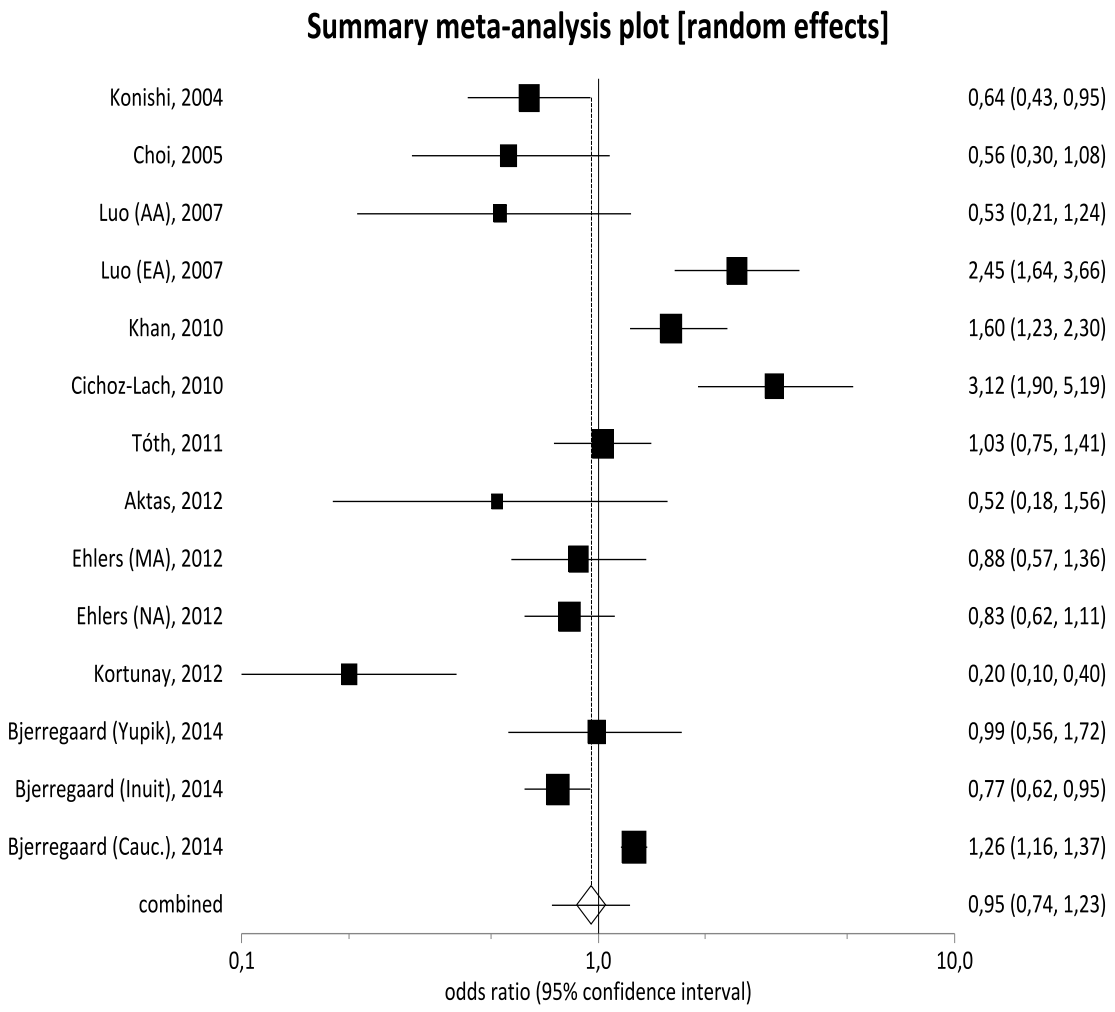


Figure 1.13
Association of the ADH1C*1/1 genotype with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (EA=European American, AA=African American, MA=Mexican American, NA=Native American, Cauc.=Caucasian).

Results of the ADH1C*1/1 (1) meta-analysis: The meta-analysis implied no significantly decreased or increased risk for alcohol dependence by being carrier of the ADH1C*1/1 gene variant (OR=0,95; 95% CI=0,74-1,23). Thus the combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,20 to

3,12. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=87,1%). This led to the application of a random effects model (DerSimonian-Laird).

There doesn't seem to be any effect of the ADH1C*1/1 genotype for the development of alcohol dependence. Lots of odds ratios confirm that (Bjerregaard (Yupik) et al., 2014; Bjerregaard (Cauc.) et al., 2014; Ehlers (MA) et al., 2012; Ehlers (NA) et al., 2012; Tóth et al., 2011). Other studies claim a significantly positive or negative association between the SNP and alcoholism (Kortunay et al., 2012; Konishi et al., 2004; Cichoz-Lach et al., 2010; Luo (EA) et al., 2007). Although the majority of the studies reports an OR less than 1 or the highest at about 1, there are two studies dealing with odds ratios even higher than 2. Cichoz-Lach et al. (2010) states that carriers of the ADH1C*1/1 are more vulnerable to alcoholism, starting to drink at a lower age than people with another genotype. In the European American population of the study by Luo et al. (2007) there also results a vulnerability to alcohol dependence by being carrier of two ADH1C*1 alleles that is more than twice as high as the risk for people not being carrier for the homozygous allele of this ADH1C SNP (OR=2,45; 95% CI: 1,64-3,66). On the other hand Kortunay et al. (2012), Aktas et al. (2012), Konishi et al. (2004) and Choi et al. (2005) report a protective impact of the ADH1C*1/1 genotype against alcoholism. The clearest association of ADH1C*1/1 and a protection from alcohol dependence is given in Turkish populations (Kortunay et al., 2012; Aktas et al., 2012). There is a positive association between alcohol dependence and the ADH1C*1/1 genotype in Caucasians reported (Bjerregaard et al., 2014; Cichoz-Lach et al., 2010). Tóth et al. (2011) didn't find an association in another Caucasian sample. Altogether there is no combined effect given that states a significant meaning of this homozygous polymorphism in the becoming of alcoholism.

Discussion of the ADH1C*1/1 (1) meta-analysis: Together with the results from the ADH1C*2/2 meta-analysis, we can assume that the polymorphisms of the ADH1C gene do not alter the risk of becoming alcohol dependent in a clinically significant manner.

We checked if publication bias exists by using a funnel plot and Egger's regression test:

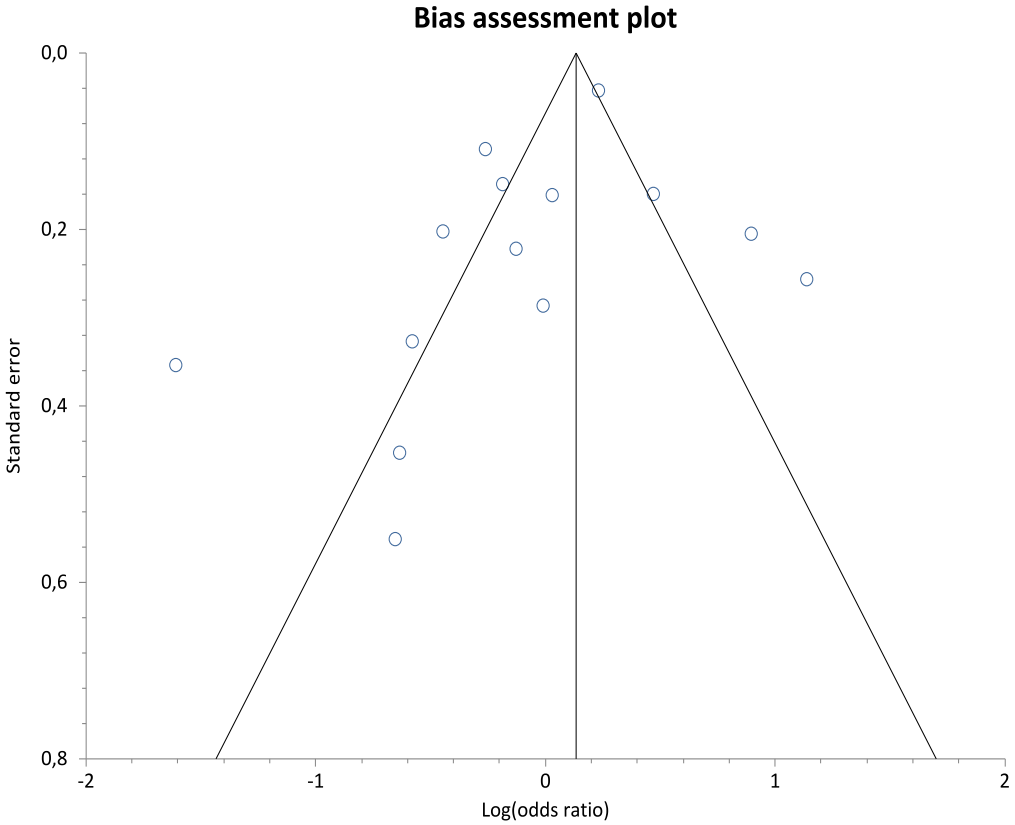


Figure 1.14
The funnel plot is showing log(OR) and standard error for the association of ADH1C*1/1 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,19.

While the 95% CIs were all quite small which stands for adequate group sizes, several studies didn't meet Hardy Weinberg Equilibrium in the control group. That's why we created a second forest plot without these studies to investigate the issue if the results might be blurred by factors different from allelic inheritance:

Summary meta-analysis ADH1C*1/1 (2)

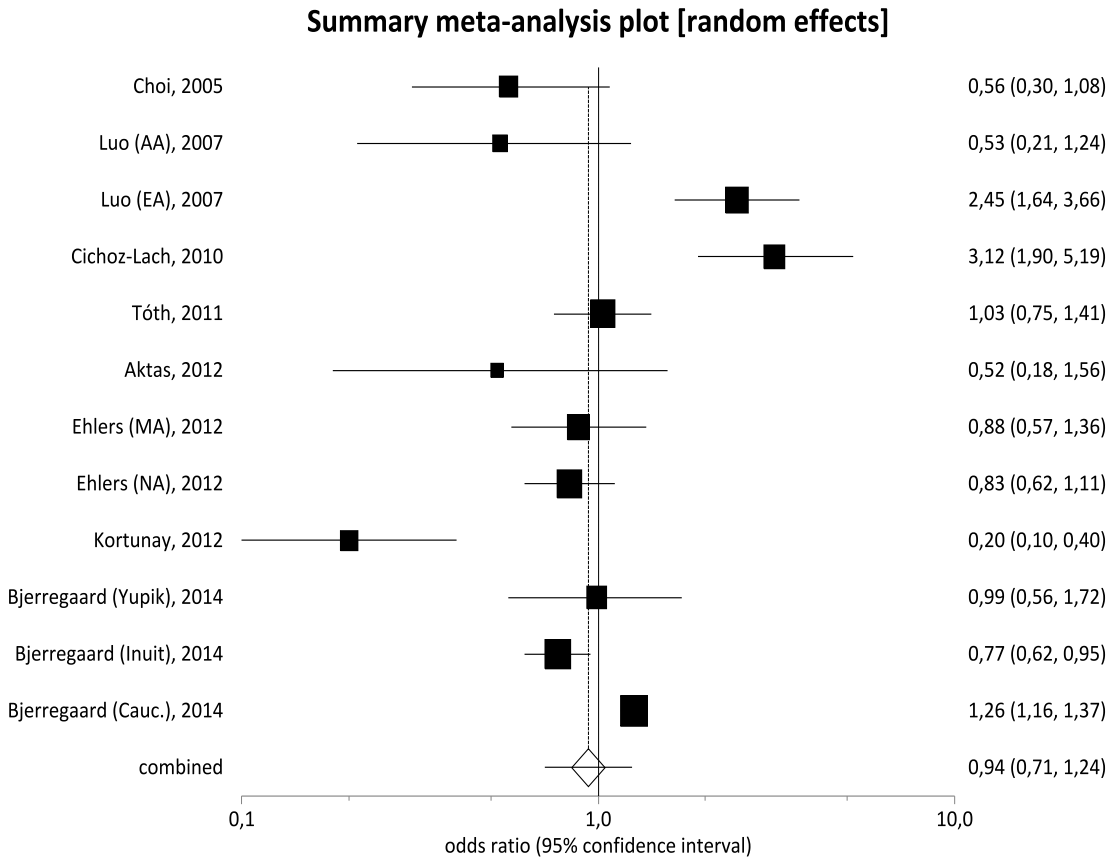


Figure 1.15
Association of the ADH1C*1/1 genotype with alcohol dependence after excluding several studies because they didn't meet HWE in the control group. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the ADH1C*1/1 (2) meta-analysis: The meta-analysis implied no significantly decreased or increased risk for alcohol dependence by being carrier of the ADH1C*1/1 gene variant (OR=0,94; 95% CI=0,71-1,24). Thus the combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,20 to 3,12. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=87,5%). This led to the application of a random effects model (DerSimonian-Laird).

Discussion of the ADH1C*1/1 (2) meta-analysis: Still there is no association between the ADH1C*1/1 genotype and alcoholism recognisable. This genotype doesn't seem to influence the likeliness of becoming alcohol dependent at all and thus affiliates the homozygous polymorphism ADH1C*2/2 which also does not alter the risk of regular ethanol consumption (**Figure 1.11**).

As a sensitivity analysis we were looking for publication bias in the following figure:

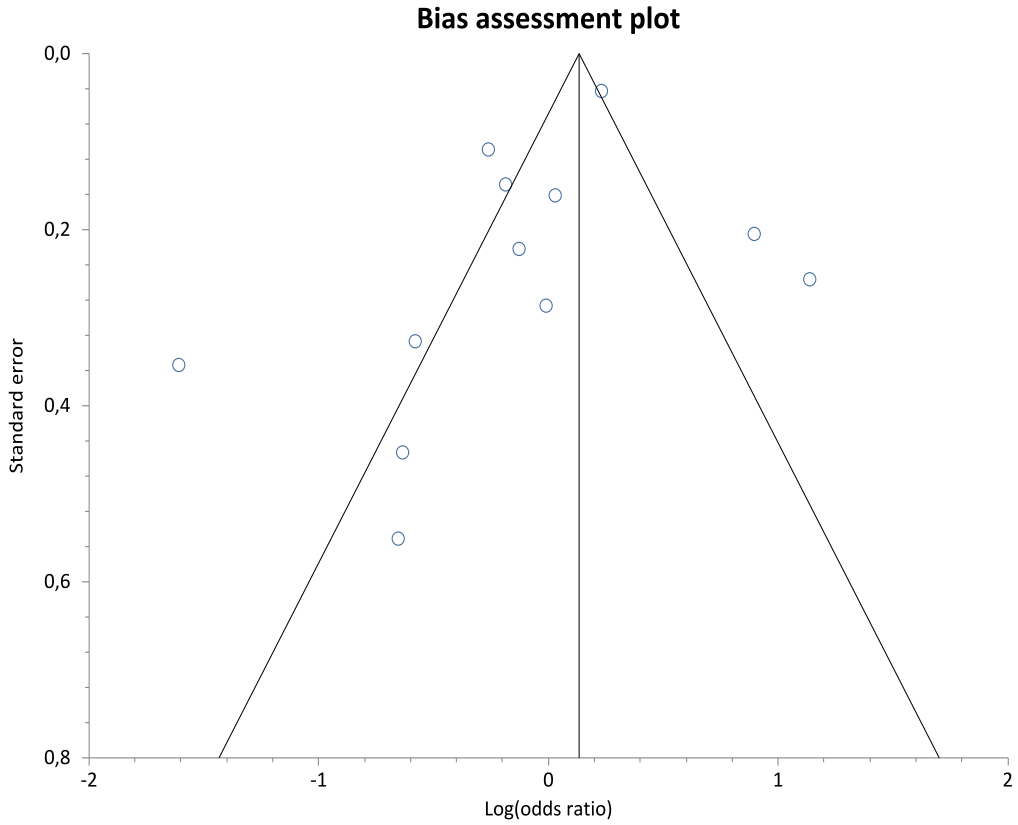


Figure 1.16
The funnel plot is showing log(OR) and standard error for the association of ADH1C*1/1 with alcohol dependence after just including the studies that met HWE in the control group. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,22.

Several studies concentrated on other polymorphisms apart from the common ADH1B and ADH1C SNPs. In contrast to these two, the other mutations have been suspicious in some populations but there is mostly not enough data given to make a clear comparison between these polymorphisms in a meta-analysis. It will be subject of new research programs to evaluate these single results and only after that it will be possible to make a thesis about the reliability of this data. Moreover there are examinations that analyzed combinations of mutations to clarify if combined alleles increase the risk of alcoholism in an outstanding way or not. Again there is some data but still not enough to compare these results in a meaningful manner.

For example the ADH4 gene cluster has been taken into consideration to cause predispositions for becoming alcohol dependent more. The subunit, encoded by the ADH4 gene, can also influence the kinetics of the enzyme, even though its importance is not as big as the one of the subunit 1 for the ethanol oxidation (Luo et al., 2005). Several studies analyzed the meaning of the ADH4 allelic polymorphisms for alcohol dependence. Luo et al. (2005) characterized 6 genotypes of the ADH4 associated with alcohol dependence after examining 365 unrelated healthy controls and 560 unrelated cases. Moreover Luo et al. (2006) continued the study started the year before and found 7 SNPs spanning the ADH4 gene considered to be important for an association with alcoholism. They characterized two SNPs showing the greatest degree for an association. On the one hand this was SNP2 (rs1042363) at exon 9 and on the other hand the SNP6 (rs1800759) at the promoter region was conspicuous. That specifies earlier studies describing association data for a region on chromosome 4 encoding information for the ADH4 with alcoholism (Edenberg et al., 1999).

Again on chromosome 4q another study analyzed 110 SNPs across 7 ADH genes on chromosome 4q22 by using the pedigree disequilibrium test (Edenberg et al., 2006). The strongest evidence was given by the ADH4 gene that affected the rate of alcoholics significantly. Some SNPs in the ADH4 gene cluster extend into the intergenic region between ADH4 and ADH5 gene. After all 12 SNPs spread all over the alleles for the ADH were associated with alcohol dependence by altering the pharmacokinetics of the ethanol metabolizing enzyme. The SNP with the greatest association (p=0,004) was rs4148886 which is part of the ADH4 sequence.

In a German study that took part in Munich, Mainz and Regensburg, there were 1622 inpatient subjects and 1469 controls analyzed. Patients from the addiction treatment were included (Preuss et al., 2011). The subjects were genotyped in a German-Polish multicenter sample of clinically well characterized alcohol dependent individuals and healthy controls. The participants' group could be divided into 454 patients from Munich, 253 patients from Mainz, 100 patients from Szczecin (Poland) and 815 patients from Regensburg. All subjects underwent several examinations including an assessment to clarify general personal characteristics and psychiatric disorders as schizophrenia or depression. After that the participants were genotyped by standard methods such as PCR and RFLP. The results particularly focused on 2 SNPs that should be associated with alcoholism. These were rs1800759 and rs1042364 which are both located on chromosome 4q. They found altered frequencies in drinking caused by the genetic variants that encode for different ADH4 subunits hence emphasizing the role of this isoenzyme in the development of alcohol dependence.

So the results of that study by Preuss et al. (2011) confirm those of previous studies (Edenberg et al., 2006, Guindalini et al., 2005) through characterizing two SNPs in the ADH4 sequence that are responsible for an altered risk of becoming an alcoholic compared to the wildtype alleles of the ADH4. Still there are some limitations on that study. For example the alcohol dependence characteristics of the participants were obtained by interviews and subjectively retrospective memories of the patients. Moreover the group of participants was quite heterogeneous and although they tried to filter out the personal factors, there are still many influences caused by the individual characteristics of a single person that might affect the result and the information given. Thus although these results meet those of older studies, it is still necessary to question these numbers and facts because there are certain environmental and personal factors that can increase or decrease the risk of alcoholism that have nothing to do with the actual examined aspect, the genetic mutations in the ADH gene cluster, in this case especially the polymorphisms in the ADH4 gene which are basically reduced to two SNPs, rs1800759 and rs1042364.

However the number of studies dealing with the ADH4 polymorphisms for the risk of alcoholism and giving useful data was not high enough to compare the findings in a meta-analysis.

Not just the SNPs in the ADH1B, ADH1C or ADH4 sequences have been suspicious. As we mentioned above there are 7 ADH genes that theoretically all can be responsible for an increased or decreased likeliness of becoming alcohol dependent by altering the metabolizing rate of the alcohol dehydrogenase. A quite old study already explained the variety of mutations that can lead to the problem of a slower degradation of ethanol which increases the risk of consuming higher amounts of alcohol due to missing side effects or hangover symptoms. That study focused on ADH2, ADH3 and ALDH2 loci on the DNA that might influence the metabolism of ethanol. Participants were alcoholic and non-alcoholic Chinese men living in Taiwan (Thomasson et al., 1991). The scientist used leukocyte DNA for genotyping the individuals who were all belonging to the Tri-Service General Hospital in Taipei. Students, physicians and laboratory stuff of the National Defense Medical Center formed the control group. As a result they could reveal that alcoholics had much lower frequencies of ADH2*2, ADH3*1 and ALDH2*2 alleles than non-alcoholics. Especially the ADH2*2 and ALDH2*2 polymorphisms were found to be less represented in alcoholic subjects. Those SNPs produce isoenzymes that elevate the acetaldehyde levels at least transiently and cause uncomfortable side effects that limit the alcohol consumption (Thomasson et al., 1991). So the ADH3*1 and ADH2*2 alleles are supposed to produce a fast-metabolizing isoenzyme that can act protectively against the development of alcohol dependence as it leads to higher concentrations of acetaldehyde. What is more they analyzed the dependence of these polymorphisms from each other to clarify if there is a certain combination highly prevalent. Thomasson et al. (1991) realized that the ADH2 and the ADH3 genotypes are completely independent from the ALDH2 genotype.

ADH6 polymorphisms also showed significant association with alcohol dependence in a quite new study (Zuo et al., 2013). The aim was to find something out about other genes being connected with the risk of becoming an alcoholic. Therefore they analyzed 870 SNPs in the ADH genes of 3 cohorts of participants that were part of an examination of 9671 subjects for 11 different neuropsychiatric disorders including alcoholism. The three cohorts of alcoholics were ethnically divided into European American cases and controls, European Australian family-subjects with alcohol dependent probands and African American cases and controls leading to a total number of 3723 cases and 5948 controls. They used the SSAGA (Semi-structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994) to interview the participants. After cleaning genotype and phenotype data they revealed several

polymorphisms of ADH6, ADH7, as well as the already known ADH1B and ADH1C alleles that were associated with an AD but not with psychiatric diseases. In contrast to that other authors could draw a link between ADH variants and neuropsychiatric disorders, for example the Parkinson disease (Buervenich et al., 2005). The results delivered new insight as they described association with ADH6 polymorphisms and alcohol dependence.

In another study they examined 1333 only German male in-patients with severe alcohol problems and 2168 controls also of German descent for several SNPs that might contribute to the risk of becoming an alcoholic and thus they enlarged a former study (Frank et al., 2012). The participants were considered as alcohol dependent by the Diagnostic and Statistical Manual of Mental Disorders and they met the DSM-IV criteria for alcohol dependence. The fact that alcohol dependence can be seen as a partly genetic heritability of about 40-60% (Enoch & Goldman, 2002) has been the basic for that examination as well as the idea of characterizing alcohol dependence as a disorder that ranks among the most frequent causes of global diseases (WHO, 2009). Frank et al. (2012) also detected an association between alcohol dependence and ADH isoenzyme genotypes apart from the ADH1B and ADH1C isoenzymes without giving specific information about their findings.

These SNPs in ADH genes apart from ADH1B and ADH1C taken into consideration to play a part in genetic predisposing alcoholism or genetic prevention from alcoholism are not analyzed in a sufficient number of studies. So they cannot be compared or evaluated in a meta-analysis. Some of these SNPs might be of future interest but at the moment their importance on the actual, clinical development of alcoholism is not clear so far. The studies focusing on the well-established polymorphisms deliver numbers we could use for our analysis because they are stated by many different studies examining the same thesis. The fact that other SNPs among the ADH genes can potentially alter the risk for alcohol dependence is obvious as the isoenzymes oxidize ethanol, the meaning of these polymorphisms on the total effect for ethanol degradation as well as the overall alcohol consumption still has to be evaluated by future studies. The data of the studies that reported an association between alcoholism and ADH polymorphisms different from ADH1B and ADH1C is shown in **Table 5.1**.

3.2) ALDH genes

The acetaldehyde produced by the ADH must be metabolized quickly to avoid the accumulation of this toxic substance. Even little elevation of acetaldehyde levels can provoke aversive reaction and cause damage especially in the brain (Hurley et al., 2012). The aldehyde group is very reactive as known from glucose for example because of the high density of elctrons that can attack diverse gauzes and eminently the areas of the body where we find the fewest amount of defense mechanisms against radicals. Therefore it is of outstanding importance that the acetaldehyde gets oxidized to acetate and water by the acetaldehyde dehydrogenase (Abraham et al., 2011). As the alcohol dehydrogenase, the aldehyde dehydrogenase is also dominantly located in liver cells. The enzyme is assembled of subunits too, leading to slightly different amino acid sequences in each of the different isoenzymes. The most important isoenzymes are ALDH1A, ALDH1B and ALDH2 (Hurley et al., 2012). They are sharing about 70% of their amino acids but differ particularly in their locus of function. Whereas the ALDH1B and ALDH2 are both located in the mitochondria, the ALDH1A works in the cytosol of the liver cells (Jackson et al., 2013). The ALDH2 isoenzyme is known as the one with the highest catalytic activity (Hurley at al., 2012). Therefore its modifications by genetic polymorphisms are of special interest because they are causing the biggest impact on acetaldehyde metabolism. The genetic information for the ALDH2 isoenzyme is located on chromosome 12q24.2-q24.12 (Zhang et al., 2014). For heavy drinkers it is useful to have hardly any withdrawal symptoms and to benefit from comfortable effects of alcohol consumption as long as possible. That's the reason why fast metabolizing alcohol dehydrogenases do have a protective effect on alcoholism. They shorten the time of befuddlement and good feeling and increase the time suffering from negative side effects such as headache, brackishness and other withdrawal symptoms caused by the availability of acetaldehyde in the peripheral blood (Ehlers et al., 2012). If the acetaldehyde level is responsible for the protective impact on individuals when talking about alcoholism, it becomes clear that an isoenzyme of the ALDH, which works more slowly than the others, takes in a protective part towards alcoholism by enlarging the time of acetaldehyde exposure. Indeed the probably best protection given by the enzymes that are involved in alcohol degradation consists of a fast metabolising ADH and a slowly metabolising ALDH

(Konishi et al., 2004). In contrast to that a slow ADH and a fast ALDH isoenzyme lead to the exact opposite. They are abetting the development of alcohol dependence by reducing negative side-effects of ethanol consumption.

As already mentioned the ALDH2 has the biggest meaning in the oxidation of acetaldehyde which is why there was a lot of information given by several authors that we could use for our meta-analysis. While the best examined SNP (Glu504Lys) leading to the creation of the ALDH2*1 (guanine; 504Glu) brings the development of an AD forward, the ALDH2*2 (adenine; 504Lys) allele has kind of a protective effect or said in an easier way, the ALDH2*1 allele leads to an isoenzyme that works faster than the isoenzyme encoded by the ALDH2*2 allele (Jo et al., 2007). We collected the data given by the studies we could include because they complied with our criteria and conducted a statistical analysis for the homozygous carriers of either the ALDH2*1 or the ALDH2*2 allele. By analyzing the homozygous carriers, we hoped to achieve significant results that can also be transferred in a less significant manner to heterozygous allele carriers of this isoenzyme.

The allele frequency of the fast metabolizing mutation of the ALDH2*1/1 gene (guanine/guanine) is supposed to be quite low in Asian populations in comparison to other ethnic groups (Chen et al., 1999). So in particular the control groups do not reveal such a high number of members being carrier for the ALDH2*1/1 polymorphism. Furthermore about half of East Asian populations even seem to be bearer of the ALDH2*2 allele that protects them from alcohol dependence (Chen et al., 1999). This number however can be questioned by the data we gained from analyzing the included studies. Additionally in contrast to other ethnic groups, the Asians deliver information about an ALDH1 deficiency which slows down the acetaldehyde degradation as well, even if the ALDH1 has a smaller meaning in the metabolism of the substrate. To become aware of that fact, they included DSMIII-R criteria (American Psychiatric Association, 1987) cases at the mean age of about 40 years. After gaining their DNA from leukocytes, they calculated disparities in alleles. The results are shown below in the graphics.

182 Chinese and Indian patients who underwent treatment for alcoholism and 184 controls from Singapore took part in the case-control study by Tan et al. (2010). The cases had been identified by alcohol use disorder identification test (AUDIT) and severity of alcohol dependence questionnaire (SADQ). The mean age of the groups did not differ immensely

(cases: 45 years; controls: 34 years). Afterwards the examiners genotyped the participants with PCR and RFLP. Chi-square test was performed to determine if the distribution of genotypes was in accordance to HWE and to clarify the allocation between the two groups. They could link the ADH1B*2 and the ALDH2*2 alleles with a protection from alcohol dependence by counting the carriers for these alleles in the two groups and finding out that there are much higher amounts of these alleles in the controls. The direction of the association was confirming the idea of a slow aldehyde dehydrogenase lowering the risk for alcohol dependence (ALDH2*2/2: OR=0,11; CI=0,003-0,814 for Chinese). Indian samples could not be analyzed because there were no homozygous carriers for the ALDH2 allele found. By regarding the MAFs, it became obvious that there exists a higher frequency for the protective allele of the aldehyde dehydrogenase gene in Asian populations, especially in the control groups in comparison to other ethnic groups, for example European Americans (Luo et al., 2007). The effect was strong in the Chinese population in which only one of the ALDH2*2 strap was alcohol dependent meeting the DSM IV criteria. The frequency of homozygous ALDH2*2 positive Chinese was about 30% of the examined individuals in the control group compared to only 18% being carrier of the ALDH2*2 allele in the control sample of the study by Luo et al. (2007) that dealt with European American subjects or Konishi et al. (2004) that dealt with Mexican Americans and had only 1% of the participants in the control group who were carriers for this protective allele.

A significantly lower frequency of the ALDH2*2 allele in Asian patients (12%) than in controls (28%) was found in a study investigating the genetic polymorphisms of the acetaldehyde metabolising enzyme ALDH2. The examined groups consisted of 100 trauma patients that headed up in the emergency department after excessive alcohol consumption and 98 age-matched controls (Tseng et al., 2007). The ALDH2*2 /2 genotype was not found in any of the cases thus it couldn’t be used for a statistical analysis.

Chao et al. (2003) was examining the meaning of the ALDH2 polymorphisms in a population from Taiwan. Therefore they included 361 cases of alcohol dependence and 280 controls in their study. Not even one single alcohol dependent participant was homozygous carrier for the ALDH2*2 allele, during 15 members of the control sample had the homozygous form of this base exchange from guanine to adenine leading to the amino acid Lys. About a quarter of the whole control population was carrier of the ALDH2*2 allele. In contrast to that the

study by Ehlers et al. (2012) that was dealing with ALDH2*1/1 genotypes only. In the whole study there was no carrier of the ALDH2*2 allele at all. They examined Mexican as well as Native Americans. On the other hand the study by Bjerregaard et al. (2014) that analysed three different ethnic groups had 100% homozygous ALDH2*2 carriers in any of the populations. The findings of these two studies can be questioned because in particular the very high population samples in the study of Bjerregaard et al. (2014) are very unlikely to contain only homozygous carriers and not even a single heterozygous carrier of the ALDH2*2 allele. Every other study considers the ALDH2*2 allele to be the less common one, so it seems to be very unlikely that this study is dealing only with ALDH2*2/2 genotypes. Anyway these studies couldn't be included in any statistical analysis, thus not altering the combined effect of the odds ratios and additionally we didn't consider the study by Bjerregaard et al. (2014) in our interpretation of the given results

Chen et al. (1999) examined two groups of Asian subjects from two different treatment institutions in Taipei, the Tri-Service General Hospital in Taipei and the Taipei City Psychiatric Center. The alcohol dependent subjects all together met DSM-IIIR criteria. The examiners were looking after the meaning of the polymorphisms in ADH2, ADH3 and ALDH2. Concerning the ALDH2*2/2 genotype, they also couldn't collect one single individual with this genotype that was alcohol dependent at the same time. On the other hand there were several persons in the control group that were carrying this allele in a homozygous manner.

We collected the data given by the studies included dealing with ALDH2*2/2 (adenine/adenine; 504Lys/504Lys) genotypes and alcohol dependence and summed the numbers up in **Table 2.1**:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Shen (Chin.)	1997	China	0	2	0,02	0,23	0,67	-
Shen (Korean)	1997	China	0	3	0,05	0,36	0,03	-
Chen (TSGH)	1999	Taiwan	0	23	0,09	0,24	0,036	-

Chen (TCPC)	1999	Taiwan	0	23	0,1	0,24	0,036	-
Lee	2001	Korea	1	4	0,03	0,25	0,87	0,14 (0,003-1,48)
Chao	2003	Taiwan	0	15	0,12	0,25	0,383	-
Konishi	2004	USA	0	1	0	0,01	<0,001	-
Tseng	2007	Taiwan	0	8	0,12	0,28	0,88	-
Tan (Chin.)	2010	Malaysia	1	11	0,07	0,3	0,8	0,11 (0,003-0,81)
Tan (Indian)	2010	Malaysia	0	0	0	0,01	0,91	-
Shin	2010	Korea	2	9	0,1	0,17	0,38	0,75 (0,07-3,76)
Guo	2010	China	1	5	0,02	0,08	0,03	0,18 (0,004-1,63)
Ehlers, MA	2012	USA	0	0	0	0	<0,001	-
Ehlers, NA	2012	USA	0	0	0	0	<0,001	-
Bjerre-gaard (Yupik)	2014	Denmark	69	3631	0	0	<0,001	-
Bjerre-gaard (Inuit)	2014	Denmark	531	3631	0	0	<0,001	-
Bjerre-gaard (Cauc.)	2014	Denmark	1200	3631	0	0	<0,001	-

[a]Number of cases being carrier for the ALDH2*2/2 genotype

[b]Number of controls being carrier for the ALDH2*2/2 genotype

[c]Minor allele frequencies for the populations' ALDH2*2/2 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the ALDH2*2/2 genotype and alcohol dependence

Table 2.1
Characteristics of the studies dealing with carriers for the homozygous ALDH2*2/2 genotype given by author, year, country, number of ALDH2*2/2 cases and controls, minor allele frequencies, HWE and odds ratios with confidence intervals (Cauc.=Caucasian, MA=Mexican American, NA=Native American, Chin.=Chinese, TSGH=Tri-Service General Hospital in Taipei, TCPC=Taipei City Psychiatric Center).

Table 2.1 reveals that the ALDH2*2 allele is much less frequent than the wildtype, the ALDH2*1 allele. The fact that there are nearly no cases that are homozygous carriers of this SNP, while at least some control subjects with this genotype could be included in respective study, may emphasize the protective effect of this mutation against alcoholism (Shen et al., 1997; Chen et al., 1999; Chao et al., 2003; Tseng et al., 2007). The allele frequencies of this polymorphism consequently are higher in the controls than in the cases. On the other hand, this lack of individuals being alcohol dependent and homozygous carriers for the ALDH2*2 allele made it impossible to gain significant results in a meta-analysis because there is no opportunity to compare the risk of the different genotypes when there is no carrier of the SNP in any of the population samples. Bjerregaard et al. (2014) delivered surprising data that is not explainable and has to be questioned. We didn't consider this data when analyzing the meaning of the ALDH2*2/2 genotype on alcoholism.

We had to exclude some studies because of missing control groups (Yokoyama et al., 2013; Yokoyama et al., 2014; Peng et al., 2014; Vidal et al., 2004). Other studies couldn't deliver useful information because they provided the data in an idiosyncratic manner: Shin et al. (2011); Matsuo et al. (2013); Husemoen et al. (2008); Kuo et al. (2008); Lu et al. (2012). The whole articles by Zhang et al. (2014) and Ye et al. (2010) couldn't be analyzed because the articles were only available in Chinese. The study by Bjerregaard et al. (2014) hasn't been considered either for the reasons mentioned above.

The results of the studies dealing the ALDH2*2/2 genotype being prevalent in both, cases and controls, and its meaning for the development of alcoholism are summed up and presented in the Forest Plot below:

Summary meta-analysis ALDH2*2/2

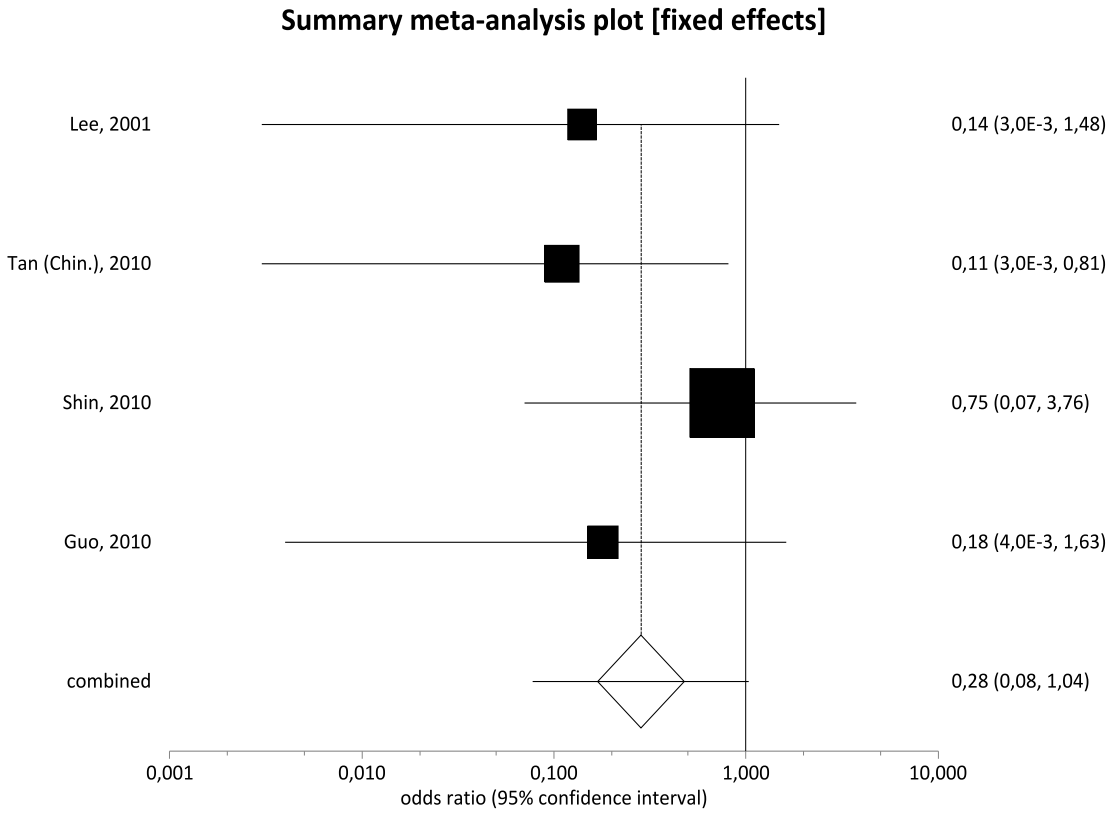


Figure 2.1
Association of the ALDH2*2/2 genotype with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (Chin.=Chinese).

Results of the ALDH2*2/2 meta-analysis: The meta-analysis implied no significantly decreased or increased risk for alcohol dependence by being carrier of the ALDH2*2/2 gene variant (OR=0,28; 95% CI=0,08-1,04). Thus the combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,14 to 0,75. The Cochran Q test (P=0,65) confirmed quite little between-study heterogeneity, so did

the inconsistency test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel-method).

Every study included stated a protective effect of the ALDH2*2/2 genotype against alcoholism without delivering significant results. The fact that there were only very little amount of homozygous carriers for this mutation in most of the studies led to the problem of quite large 95% confidence intervals (Lee et al., 2001; Guo et al., 2010; Tan (Chin.) et al., 2010). What is more there are 10 populations for who we could not calculate any ratios because there were no carriers of the ALDH2*2/2 genotype at all. Most of the time there were no cases being carrier for this genotype, while at least a few control subjects were found to be homozygous carriers for this polymorphism. Also in every study we included in **Table 2.1** there were more carriers of the ALDH2*2 allele in the control sample. Asian populations generally counted higher number of ALDH2*2/2 genotypes in comparison with other ethnic groups (Shin et al., 2010; Tan et al., 2010; Guo et al., 2010; Lee et al., 2001).

Discussion of the ALDH2*2/2 meta-analysis: After all it seems that this polymorphism of the ALDH2 gene cluster that is supposed to encode for a slowly metabolizing isoenzyme (Chen et al., 1999) is quite rare in any population throughout our analysis. The lack of homozygous carriers for the polymorphism ALDH2*2 made it difficult to compare the risk of these genotypes with others. This problem occurred in several studies (for example Ehlers et al.; Konishi et al., 2004; Tan (Indian) et al., 2010; Shen et al., 1997). So this forest plot probably cannot describe the meaning of this mutation in an adequate way. We rather can consider the ALDH2*2/2 genotype to be a quite rare polymorphism that is even rarer in alcohol dependent cases than in non-affected controls. So it is likely that this SNP encoding for a more or less inactive isoenzyme has an impact on the drinking behaviour. The effect might even be that strong that there is a complete lack of carriers for the ALDH2*2/2 genotype being alcohol dependent. This might be caused by the uncomfortable impact of alcohol consumption on the subjects' organism that is accumulating acetaldehyde for a longer time. On the other hand the total frequency of this genotype in the whole population may be that seldom, that there is no statement about its importance for alcohol dependence possible. At least what we can note is, that a meta-analysis like this is ineffectual for describing the meaning of the ALDH2*2/2 genotype for the development of alcoholism. The numbers we gain from analyzing those studies that allow a statistical analysis are not significant at all. The amount of ALDH2*2/2 carriers is just too small. We could only include 4 studies in our meta-

analysis. Therefore a second meta-analysis for this homozygous SNP, excluding studies whose control group doesn't meet HWE (Guo et al., 2010) hasn't been performed because we would just decrease the already low number of studies included. After analysing the forest plot, the meaning of the ALDH2*2/2 could possibly result in a protection from alcoholism but cannot be described in a forest plot but rather by just evaluating the tiny amount of alcohol dependent cases being carrier for this genotype. The higher prevalence in control samples can point out that there results a protective effect on the development of alcoholism by being carrier of the ALDH2*2 allele. Furthermore we support the findings that the ALDH2*2 allele is more frequent in Asians than in other populations, such as European American, Caucasian, Native American (Amamoto et al., 2002). The studies dealing with Asians mostly counted more than 20% being carrier for the ALDH2*2 allele, during examinations of other ethnic groups had less carriers of this allele (Ehlers et al., 2012; Konishi et al., 2004). Hence Asians can be considered to be rather protected from alcohol dependence by carrying the ALDH2*2/2 genotype in a higher frequency than other ethnic groups. Still this cannot be supported by our forest plot, that is not useful to interpret the meaning of the ALDH2*2/2 genotype on alcohol dependence, but by regarding the numbers in **Table 2.1**, that suggest a negative association between the ALDH2*2/2 genotype and alcohol dependence. We also found much more studies dealing with Asian participants and the ALDH2*2 allele than studies with the ALDH2*2 allele in other ethnic groups, which might be result of the low availability of that allele in populations other than Asian. What is more it is very likely that already one mutated allele leading to the heterozygous ALDH2*1/2 genotype is enough to result in a significant protection from alcoholism as the way of inheritage of the ALDH2 gene is thought to be autosomal dominant with the ALDH2*2 allele to be the dominant one leading to an inactive aldehyde dehydrogenase (Jo et al., 2007). We compared the ALDH2*1/1 genotype to the heterozygous ALDH2*1/2 and the ALDH2*2/2 genotype in **Figure 2.3**.

We checked if there exist publication bias by using a funnel plot and Egger's regression test:

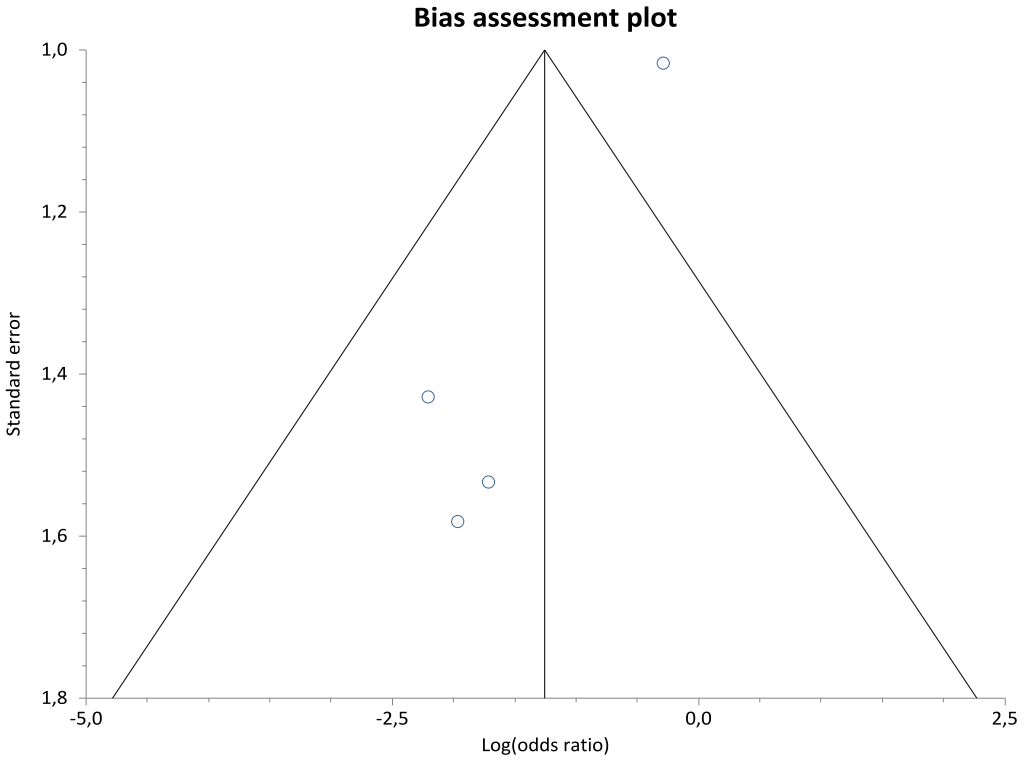


Figure 2.2
The funnel plot is showing log(OR) and standard error for the association of ALDH2*2/2 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,06.

As we already mentioned, the statistical analysis including odds ratios and 95% confidence intervals is not very helpful for interpreting the meaning of the ALDH2*2/2 genotype because there are not enough carriers of that genotype to gain significant results. In particular the case samples reported an absolute lack of carriers. We could only include 4 studies because the others didn't even count one single carrier of this genotype in the case groups. The small amount of ALDH2*2/2 carriers is emphasized by the fact that some studies list them together with the heterozygous ALDH2*1/2 genotypes (Lu et al., 2012), which

made it impossible to include the results in our meta-analysis dealing with the effect of the homozygous genotypes for alcohol dependence but also stresses the fact that the ALDH2*2 allele is thought to be dominant. The absolute lack of ALDH2*2/2 carriers in the case samples might emphasize the importance of an inactive ALDH2 isoenzyme for a protection from alcohol dependence. Unfortunately it is not suitable for a meta-analysis of this kind which requires predisposed cases and controls as well to gain significant results. However the studies that we could include in the forest plot stated a decreased likeliness of becoming alcohol dependent when being carrier of the ALDH2*2/2 genotype, even if they couldn't deliver significant results.

The ALDH2*1 (guanine; 504Glu) allele has also been subject of several case-control and cohort studies examining the importance of ALDH2 polymorphisms on the risk for alcohol dependence. The isoenzyme which is encoded by the ALDH2*1 allele is supposed to have a much higher efficiency than the one encoded by the ALDH2*2 allele (Tan et al., 2010). Therefore the much more frequent allele, the ALDH2*1 allele, is probably involved in a predisposition for alcohol dependence as it lowers the time the organism suffers from the consequences of a high acetaldehyde level in the blood (Guo et al., 2010). So we also analysed the meaning of the ALDH2*1 wildtype allele in a homozygous form for an association with alcohol dependence, keeping in mind, that the ALDH2*2/2 genotype was very rare, especially in the case samples, however the ALDH2*2 allele is thought to be dominant (Jo et al., 2007) which means that already the heterozygous genotype may suffer from an inactive aldehyde dehydrogenase.

We collected the data given by the studies included dealing with ALDH2*1/1 (guanine/guanine; 504Glu/504Glu) genotypes and alcohol dependence and summed the numbers up in **Table 2.2**:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Shen (Chin.)	1997	China	50	28	0,02	0,23	0,67	17,37 (3,78-164,2)
Shen (Korean)	1997	China	49	17	0,05	0,36	0,03	15,3 (5,2-52,93)
Chen (TSGH)	1999	Taiwan	236	304	0,09	0,24	0,036	1,79 (1,34-2,42)
Chen (TCPC)	1999	Taiwan	114	304	0,1	0,24	0,036	3,34 (2,1-5,47)
Lee	2001	Korea	102	35	0,03	0,25	0,87	13,8 (5,1-44,23)
Chao	2003	Taiwan	269	154	0,12	0,25	0,383	2,38 (1,69-3,39)
Konishi	2004	USA	200	249	0	0,01	<0,001	-
Tseng	2007	Taiwan	77	51	0,12	0,28	0,88	3,07 (1,6-5,99)
Tan (Chin.)	2010	Malaysia	67	56	0,07	0,3	0,8	6,38 (2,76-15,96)
Tan (Indian)	2010	Malaysia	103	78	0	0,01	0,91	2,64 (0,13-157,3)
Shin	2010	Korea	57	160	0,1	0,17	0,38	2,32 (1,12-5,22)
Guo	2010	China	375	301	0,02	0,08	0,03	7,61 (3,5-18,9)
Ehlers (MA)	2012	USA	126	309	0	0	<0,001	-
Ehlers (NA)	2012	USA	434	357	0	0	<0,001	-
Bjerre-gaard (Yupik)	2014	Denmark	0	0	0	0	<0,001	-

Bjerre-gaard (Inuit)	2014	Denmark	0	0	0	0	<0,001	-
Bjerre-gaard (Cauc.)	2014	Denmark	0	0	0	0	<0,001	-

[a]Number of cases being carrier for the ALDH2*1/1 genotype

[b]Number of controls being carrier for the ALDH2*1/1 genotype

[c]Minor allele frequencies for the populations' ALDH2*1/1 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the ALDH2*1/1 genotype and alcohol dependence

Table 2.2
Characteristics of the studies dealing with carriers for the homozygous ALDH2*1/1 genotype given by author, year, country, number of ALDH2*1/1 cases and controls, minor allele frequencies, HWE and odds ratios with confidence intervals (Cauc.=Caucasian, MA=Mexican American, NA=Native American, Chin.=Chinese, TSGH=Tri-Service General Hospital in Taipei, TCPC=Taipei City Psychiatric Center).

As we can realize by the first view on the studies' data, there are much more studies for which we could calculate odds ratios and 95% confidence intervals. Of course the studies by Bjerregaard et al. (2014) and Ehlers et al. (2012) couldn't be included in the statistical analysis again because they reported a percentage of ALDH2*1/1 carriers of 100% from the whole examined populations.

We had to exclude some studies because of missing control groups (Yokoyama et al., 2013; Yokoyama et al., 2014; Irons et al., 2012; Vidal et al., 2004). Other studies couldn't deliver useful information because they provided the data in an idiosyncratic manner: Matsuo et al. (2013); Husemoen et al. (2008); Kuo et al. (2008); or they didn't reveal exact genotype numbers (Lu et al., 2012). The whole articles by Zhang et al. (2014) and Ye et al. (2010) couldn't be analyzed because the articles were only available in Chinese. The study by Chen et al. (2009) had to be excluded because it was no case-control or cohort study.

The results of the studies dealing the ALDH2*1/1 genotype and its meaning for the development of alcoholism are summed up and presented in the Forest Plot below:

Summary meta-analysis ALDH2*1/1 (1)

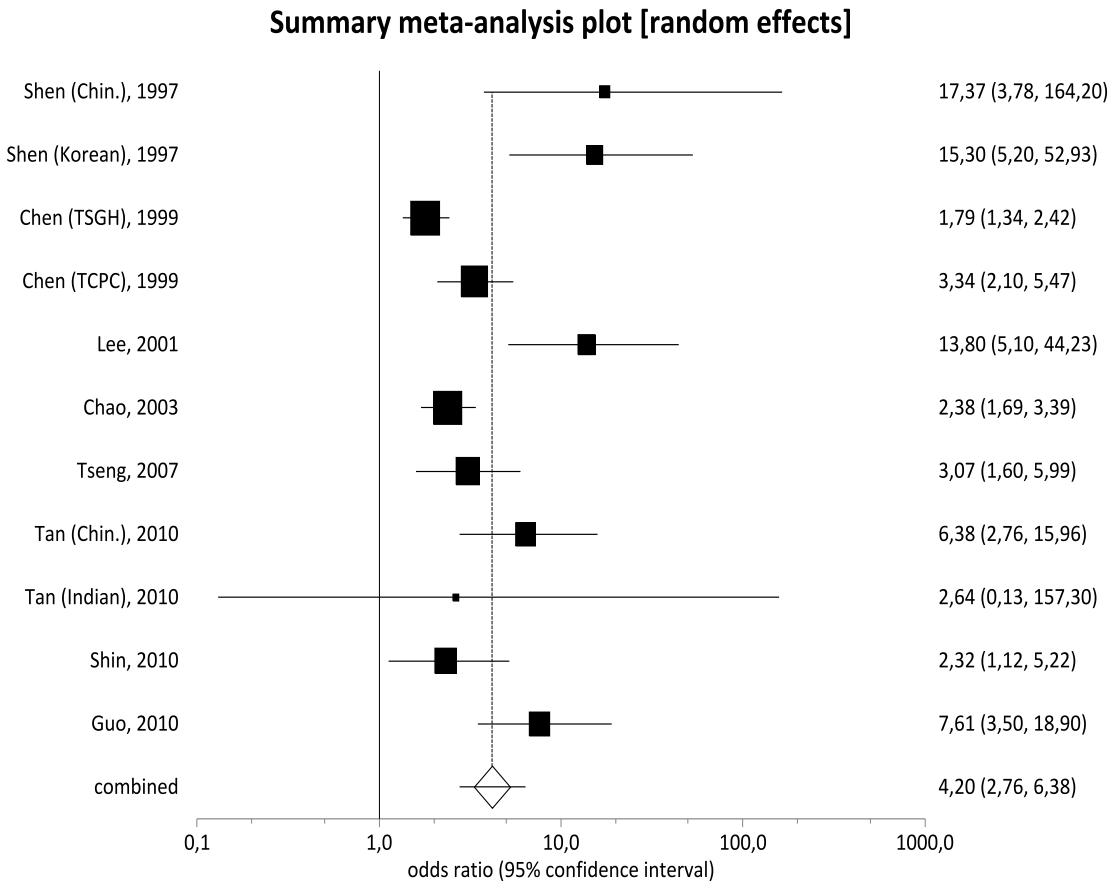


Figure 2.3
Association of the ALDH2*1/1 genotype with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis (Chin.=Chinese, TSGH=Tri Service General Hospital Taipei, TCPC=Taipei City Psychiatric Center).

Results of the ALDH2*1/1 (1) meta-analysis: The meta-analysis implied a significantly increased risk for alcohol dependence by being carrier of the ALDH2*1/1 wildtype in comparison to ALDH2*1/2 and ALDH2*2/2 genotypes (OR=4,20; 95% CI=2,76-6,38). Thus the combined effect revealed a significant association between the SNP and alcoholism. The

individual odds ratios ranged from 1,79 to 17,37. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=74,7%). This led to the application of a random effects model (DerSimonian-Laird).

The forest plot describes that the ALDH2*1/1 genotype has a significantly bringing forward effect towards alcoholism. Any of the studies we could include in our meta-analysis reported positive association between this genotype and alcohol dependence. What becomes obvious is that there are just studies with Asian participants. The studies with other ethnic groups did not deliver results that made it possible to include them in our meta-analysis. The reason for this lay in the very low MAFs of carriers of the ALDH2*2 allele. Thus there is nearly no genotype except the ALDH2*1/1 prevalent and no calculation of ORs possible. In contrast to that, the prevalence of the ALDH2*2 allele in Asian populations is much higher in the majority of the studies. For example Chao et al. (2003), Chen et al. (1999), Shen et al. (1997) and Tseng et al. (2007) all deal with ALDH2*2 MAFs of more than 20% in the control sample, while Konishi et al. (2004) and Ehlers et al. (2012) counted nearly no single subject with this allele in their studies with ethnic group different from Asians.

Discussion of the ALDH2*1/1 (1) meta-analysis: We also found a large number of studies with Asian participants which illustrates that the ALDH2 polymorphisms are considered to be involved in alcohol dependence in Asian populations for a long time in contrast to ADH1C polymorphisms for example, for which we couldn't find many studies dealing with Asian participants. The significant association between the ALDH2*1/1 genotype and alcohol dependence, which has to be seen in comparison to the polymorphisms at the ALDH2 gene locus, also emphasizes the role of the SNPs leading to a less vulnerable genotype towards alcoholism. The fact that the ALDH2*1/1 genotype leads to a significant increase in the risk for becoming alcohol dependent, makes clear that the dominant ALDH2*2 allele represents a protective one in the progress of alcohol dependence as already one allele decreases the risk significantly. However the amount of people being homozygous carriers for the ALDH2*2 allele was too small to make a clear statement about the vulnerability of this homozygous genotype towards alcoholism in a meta-analysis. As **Figure 2.3** outlines the increased risk of ALDH2*1/1 genotypes compared to ALDH2*1/2 and ALDH2*2/2, it is obvious that if we would create a forest for ALDH2*2/2 plus ALDH2*1/2 in comparison to ALDH2*1/1 to improve the understanding of the importance of the ALDH2*2 allele, we would gain opposite results to **Figure 2.3**. Moreover the results from the forest plot above stress the fact that the

ALDH2*2 allele is dominant as already the heterozygous genotype has a significantly decreased risk of becoming alcohol dependent compared to the ALDH2*1/1 genotype.

Although we gained significant results from **Figure 2.3**, we created a second forest plot just including studies that had a confidence interval smaller than 8 to guarantee a quite small standard deviation and a participants' sample that was big enough to have enough individuals with any of the possible genotypes for the ALDH2 gene cluster. We still included the study by Chen et al. (1999), although it wasn't meeting HWE in the control sample. But otherwise the number of studies included would be too small.

But first we checked if publication bias existsby using a funnel plot and Egger's regression test:

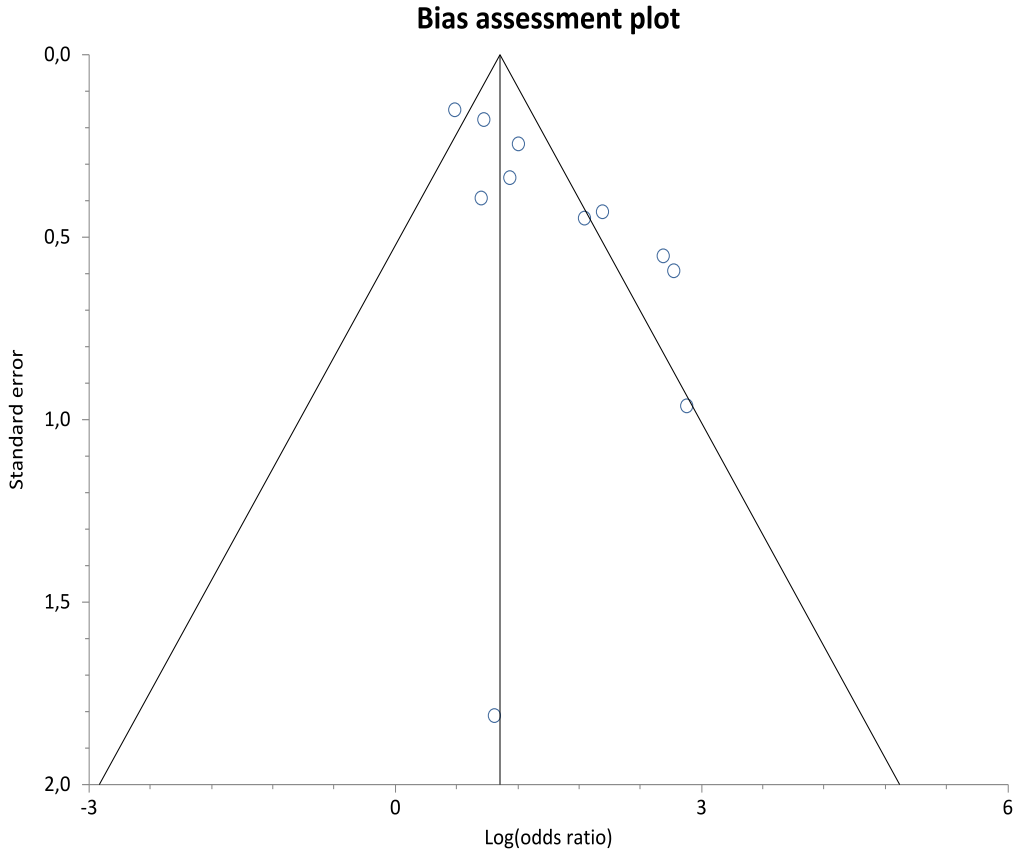


Figure 2.4
The funnel plot is showing log(OR) and standard error for the association of ALDH2*1/1 with alcohol dependence. The bias indicators showed some significant deviation from the symmetry assumption, thus indicating publication bias. Egger's regression test: P=0,005.

The funnel plot reveals that the results deviate from statistical spread, thus indicating publication bias. The problem with these results consists in the fact that in some cases there are just a few participants that are not carrier of the ALDH2*1/1 genotype. Hence we calculated very big odds ratios that cause publication bias as the funnel plot shows. The study designs however all satisfied our quality assessment being controlled, retrospective case-control studies that were allocating the subjects to the case or control group by processing either structured or semi-structured questionnaires or interviews according to DSM criteria (Guo et al., 2010; Shin et al., 2010) or collecting cases from treatment centers and controls from general healthy population (Chen et al., 1999; Tseng et al., 2007; Tan et al., 2010; Lee et al., 2001). So the problem should have to do with the allelic distributions in the studies. That's why we generated a second meta-analysis just including studies that were dealing with 95% confidence intervals that are smaller than 8, thus representing rather significant results than some of the studies in our first analysis of the ALDH2*1/1 genotype. In this case we also included the study by Chen et al. (1999) even without meeting HWE in the control group to enlarge the number of included studies that give results that deal with a useful 95% CI.

Summary meta-analysis ALDH2*1/1 (2)

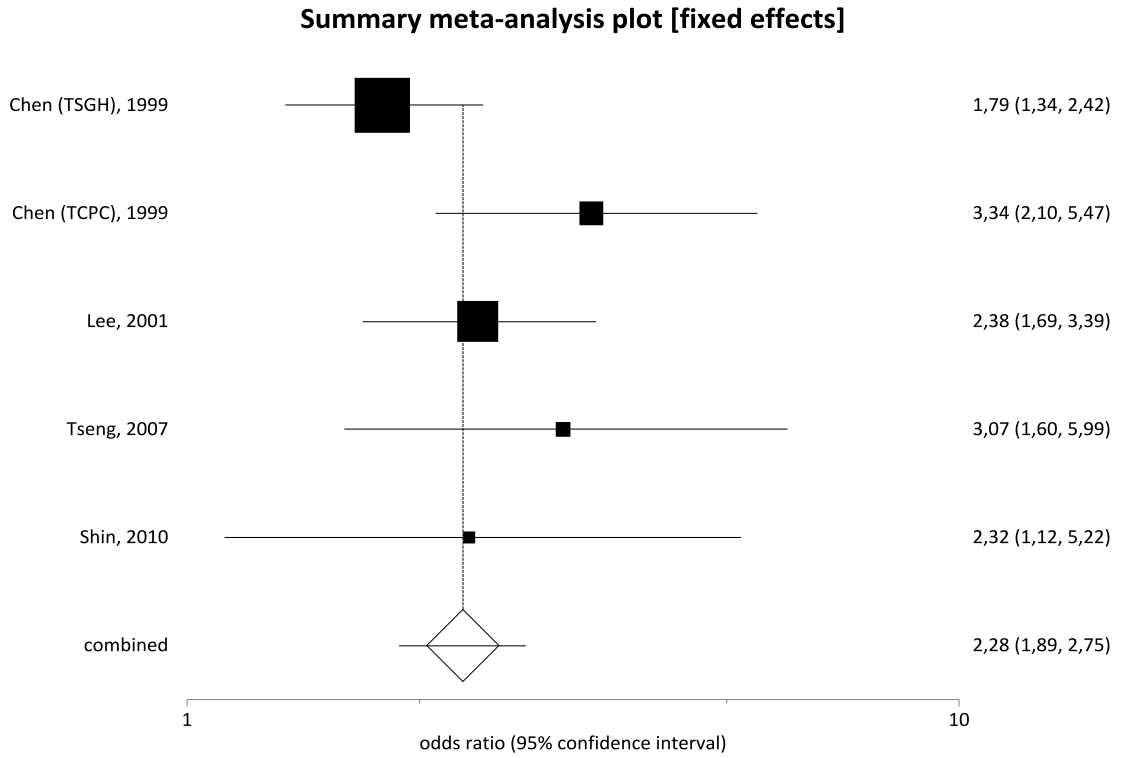


Figure 2.5
Association of the ALDH2*1/1 genotype with alcohol dependence after excluding several studies with 95% CIs that were too large to produce useful data. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the ALDH2*1/1 (2) meta-analysis: The meta-analysis implied a significantly increased risk for alcohol dependence by being carrier of the ALDH2*1/1 wildtype in comparison to ALDH2*1/2 and ALDH2*2/2 genotypes (OR=2,28; 95% CI=1,89-2,75). Thus the combined effect revealed a significant association between the SNP and alcoholism. The individual odds ratios ranged from 1,79 to 3,34. The Cochran Q test (P=0,21) confirmed quite little between-study heterogeneity, so did the inconsistency test (I^2=37,1%). This led to the application of a fixed effects model (Mantel-Haenszel method).

We just included the studies with 95% confidence intervals smaller than 8 in this second forest plot. The combined effect shows that there exists a significant association between the ALDH2*1/1 genotype and alcohol dependence. Individuals with this genotype have a 2,28

fold risk to become alcohol dependent compared with those being carrier for at least on ALDH2*2 allele.

Discussion of the ALDH2*1/1 (2) meta-analysis: The fast metabolizing acetaldehyde dehydrogenase leads to reduced hangover symptoms after alcohol consumption and thus increases the likeliness of repeated alcohol intake. Still this statement just refers to Asian populations because we could just include the studies that had also participants with the ALDH2*2 allele in sufficiently high numbers.

We created another funnel plot to clarify whether there still exist publication bias or if they derived from the studies we excluded from the second analysis:

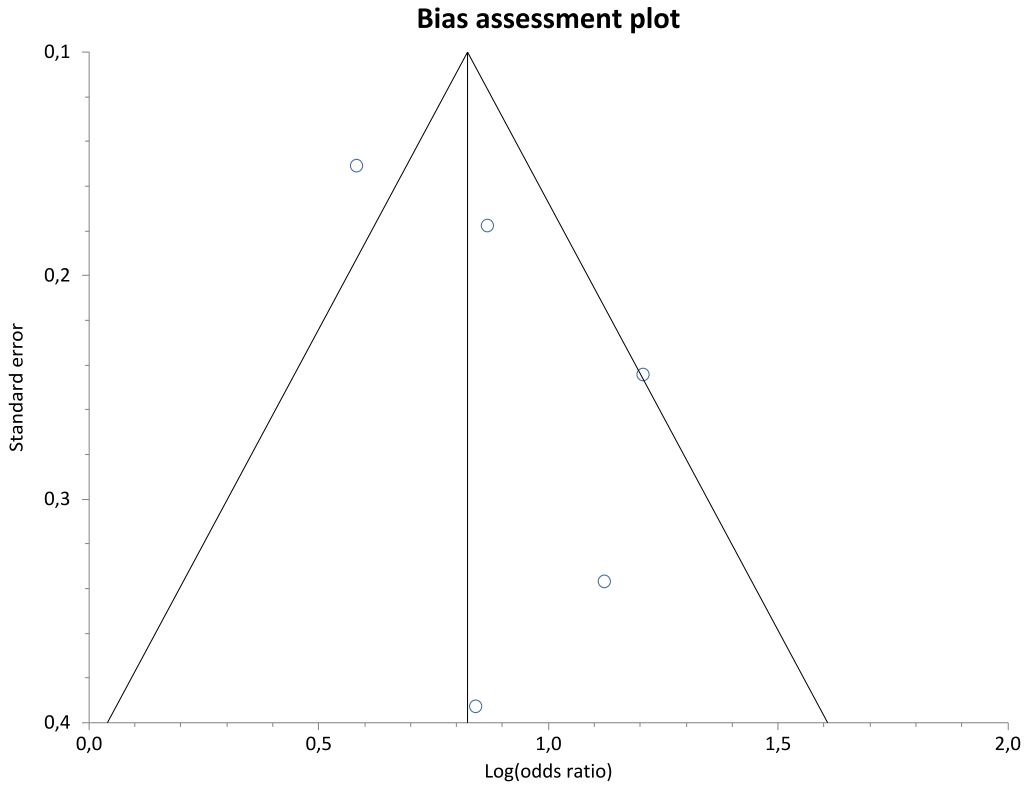


Figure 2.6
The funnel plot is showing log(OR) and standard error for the association of ALDH2*1/1 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,25.

After excluding the studies with very large 95% CIs, we could create a meta-analysis without detecting publication bias for the remaining data. Thus, although the problem of the little amount of ALDH2*2/2 persists, we could collect a few studies that delivered significant, reliable data lying in statistical spread which indicates that there is no bias influencing the results.

Altogether we can report that it is stated that the ALDH2*2 allele has a significantly protective effect on the development of alcoholism. This ALDH2*2/2 genotye is very rare however which makes it impossible to gain useful data by performing a meta-analysis. We can just assume, that this genotype represents a protection from alcoholism if we regard the minor allele frequencies in the case and control groups. Throughout all the studies the allele frequency of the ALDH2*2 allele was higher in the control sample than in the cases and also absolutely very low in the case group. This emphasizes the potential protection from alcoholism, individuals gain especially by being homozygous carriers for this rare SNP. The very common ALDH2*1/1 genotype has a significantly bringing forward effect on alcoholism for its carriers. For this wildtype allele it was much easier to perform a statistical analysis because there were much more subjects for the homozygous genotype. Nevertheless through the tiny amount of ALDH2*2/2 carriers there were also partly useless numbers for the meaning of the ALDH2*1/1 genotype for alcohol dependence. So we had to exclude lots of studies from a second forest plot. But still we achieved significant results for an association of the ALDH2*1/1 genotype and alcohol dependence. So in the end we can be sure that the polymorphisms of the gene encoding for the isoenzyme that performs the majority of the acetaldehyde oxidation, the ALDH2, have a significant impact on the risk of becoming alcohol dependent. Particularly Asian populations are characterised by a comparatively high appearance of that ALDH2*2 allele as you can see by the MAFs in **Table 2.1** and **Table 2.2**. This is also why the majority of the studies we found dealing with the ALDH2 polymorphism (Glu487Lys) and alcoholism is about Asian populations.

Although we already mentioned that the ALDH2 isoenzyme has distantly the biggest importance for the metabolism of acetaldehyde, the polymorphisms in the genes for the ALDH1A and ALDH1B variants do also alter the enzyme kinetics. This may also influence the individual drinking behaviour by increased or decreased problems after alcohol intake. However it remains unclear if the effect of the ALDH1A and ALDH1B SNPs is clinically significant. On the one hand it is reported that the ALDH1A and ALDH1B only contribute to the oxidation of acetaldehyde when ALDH2 is inactive (Hurley et al., 2012). But on the other hand there are studies drawing a connection between the allele and the conduct with alcohol. Lind et al., (2008) found an association between the allele of the cytosolic ALDH1A1 and alcoholism in a population of Europeans. They executed an analysis with 104 Finnish persons who were recruited from alcohol dependence treatment institutions and 201 controls from general population. The SNP in rs348449 was highly suspicious to influence the metabolism and thus the drinking behaviour in a significant way. However the results for this polymorphism and those of others except from ALDH2 polymorphisms stay isolated cases that cannot be assured by others and therefore it would not be expedient to include those numbers in our meta-analysis. The data of the studies that reported an association between alcoholism and rare ALDH polymorphisms different from the ALDH2 polymorphisms is shown in **Table 5.1**.

After working through the ALDH polymorphisms' impact on the actual association with alcohol dependence, it becomes clear that the ALDH2 is the best examined so far. It is located in the mitochondria of the liver cells and performs the majority of the acetaldehyde oxidation. Particularly Asian populations are characterised by a variety of studies and supposed to have a very high appearance of that ALDH2*2 allele (Chen et al., 1999; Tseng et al., 2007) in comparison to other ethnic groups as our meta-analysis confirms.

3.3) GABA-A receptor genes

The biogenic amine of the proteinogenic amino acid glutamate is called γ-aminobutyric acid (GABA). GABA is the product of the decarboxylation of glutamate`s α-carbon atom and plays a very important role as an inhibitory neurotransmitter, especially in human brains. In contrast to that, the major inhibitory neurotransmitter in the spinal cord is glycine (Fehr et al., 2006).

In general inhibitory transmitters have hyperpolarizing effects on the cell membrane, so that the regular membrane potential reaches even more negative levels. Thus the creation of an action potential becomes very difficult and there is no forwarding information. GABAergic neurons release the biogenic amine via exocytose in the synaptic gap where it binds to its specific pentameric receptors. These GABA-A receptors are ion channels for chloride anions (Cl^-) which have a quite low concentration intracellular. Consequently there results an influx of Cl^- and the membrane potential lowers, the cell membrane hyperpolarizes. Ethanol, in general, is stimulating the exocytose of GABA and thus leads to symptoms such as limited threshold, sedation and lowered cognition but also increased excitability is possible (Enoch et al., 2008).

We focused on the effects of ethanol on different types of GABA-A receptors which alleles are located on chromosome 4, 5 and 15 (Song et al., 2003). Chromosome 4 contains the genes for GABRA2, GABRA4, GABRB1 and GABRG1 subunits, the α1, α6, β2 and γ2 subunits are encoded by alleles on chromosome 5 and chromosome 15 provides information for GABRA5, GABRB3 and GABRG3. While analysing several studies, we realized that especially variations of the GABRA2 that genetic information is located on chromosome 4 p are considered having influence on the effects of alcohol and developing alcohol dependence (Edenberg et al., 2004). These receptors became of increased functional interest because animal studies could identify this α2 subunit as the primary GABA-A receptor subunit in limbic regions (McKernan and Whiting, 1996). Ethanol can have influence on the sensitivity of this subunit and thus it has a big importance for the emotions of the alcohol consumers and can be responsible for an altered drinking behaviour by inducing negative or positve

emotional effects during the organism's contact with ethanol. Moreover the α2-subunit is of major importance concerning the mediation of anxiolytic effects of benzodiazepines (Rudolph et al., 1999) which leads to the association of this medication with alcohol (Täuber et al., 2003). Also other polymorphisms of GABA-A receptors have been suspicious according to an interaction with ethanol and have been tested (Fehr et al. 2006; Anstee et al., 2013).

8 out of 9 single nucleotide polymorphisms (SNPs) in the GABRA2 gene have been identified to be associated with alcohol dependence (Edenberg et al., 2004). Edenberg et al. (2004) was focusing on the alleles located on chromosome 4p and he proved an influence on the endophenotype, in this case brain oscillations in β-frequency range when consuming alcohol. The region on chromosome 4p contains genetic information influencing the β-frequency of the EEG that also affects the risk of alcoholism because of the similarity of the neuronal mechanisms. Lots of families with at least three alcoholic members were examined and they could realize that alcohol affects several behavioural patterns such as disruption of motor coordination, anxiolysis, sedation and symptoms related to withdrawal that all have to do with an alteration in GABA transmission (Buck et al., 1996). As we already know, the α2-subunit of the GABA receptor represents a target for certain medication like benzodiazepines which are used in the treatment of alcohol withdrawal symptoms (Rudolph et al., 1999). This fact emphasizes the meaning of the α2-subunit and its genetic variations for the neuronal effects of ethanol.

A Russian population of 113 alcohol-dependent men and 100 male control subjects was genotyped for 7 SNPs on the chromosome 4p, using RT-PCR (Lappalainen et al., 2005). The findings suggest that genetic variants of the GABRA2 gene can increase the risk of alcohol dependence in the Russian population and also supports other authors that noted an important role in predisposing to alcohol dependence through SNPs in the GABRA2 gene of the GABA receptor. In this study however the homozygous SNP carriers of the GABRA2 gene were not significantly linked with alcohol dependence.

In our meta-analysis we were looking for SNPs in the GABRA2 gene region on chromosome 4p. In contrast to the polymorphisms in the ADH or ALDH gene clusters, there are several SNPs possibly linked with alcohol dependence in the GABRA2 gene. So we were collecting the SNPs that were reported by several authors when dealing with the vulnerability for

alcoholism by being homozygous carrier for mutations in the α2-subunit of the GABA receptor.

A significance of GABRG2 and GABRA6 genes for developing an alcohol dependence which leads to the importance of other polymorphisms that are responsible for the variations in the proteins which form the GABA-A receptor has also been reported (Enoch et al., 2008). These receptors have got something in common which is the fact that they undergo allosteric modulation by substances such as anesthetics, benzodiazepines, neurosteroids and ethanol. These modulations cause phenoma like ethanol tolerance, dependence and withdrawal. Especially the tolerance goes along with a generally decreased GABA receptor activation and differentially altered subunit expression (Enoch, 2008). In general, alcohol influences many mechanisms in the human brain involving neurotransmitter and neurohormone membrane receptors and receptor-gated and voltage-activated ion channels. It alters the balance between the proteinogenic amino acid glutamate and the biogenic amine GABA in favour of higher GABA agencies. To be more specific, ethanol can alter GABAergic transmission and affects through complex mechanisms pre- and postsynaptic GABA receptors. In some areas long-term ethanol consumption seems to increase mRNA expression, for example the superior frontal cortex, in other areas there is quite good evidence for a decreased mRNA expression, for example the amydala. Moreover some problems of the ethanol consumption become clear when concerning the physiological function of GABA including modulating emotion and response to stress as well as the inhibition of the hypothalamic-pituitary-adrenal axis (Enoch et al., 2008). Alcohol influences these complex systems in several ways and together with stress it stimulates the syntheses of cholesterol in the brain. This is part of the positive emotional advantages for a consumer resulting from ethanol consumption and stresses the fact that the meaning of GABA-A receptor polymorphisms is much more complex than the one of ADH and ALDH mutations which just lead to fast or slowly metabolizing enzymes. The difference in genetic information for the GABA-A receptors may cause an increased amount or functioning of GABA-A receptors in one brain region, during the other brain region counts less GABA-A receptors than the wildtype. These complex mechanisms justify that we included not just one homozygous SNP but also other homozygous SNPs of the GABRA2 gene in our meta-analysis.

To investigate the influence of the GABRA2 polymorphisms on a German population, the study by Fehr et al. (2006) analysed the drinking behaviour of carriers for particularly the GABRA2 polymorphisms of 257 German alcohol dependent participants and 88 healthy controls. They mainly focused on the middle part of that base sequence where they found an association between the polymorphisms and the risk of becoming an alcoholic. After all they reported a consistent increase of alcohol consumed in individuals with that genetic predisposition.

Another study with European American and African American participants took place in the Connecticut Health Center (Covault et al., 2008). 372 European American cases and 535 European American controls were genotyped and underwent a questionnaire so that the cases considered all met DSM-III-R criteria for alcoholism. The group of individuals consisted of people with a mean age of 41,8 years and 34% of them were alcohol dependent. In the European American group they could find a big number of carriers for an altered GABRA2 gene, in particular in the case sample, which gave evidence for an association between the polymorphisms and alcohol dependence in a European American group of participants, at least for the homozygous SNP carriers in rs279844.

Clear results in form of no association at all were delivered by a study with 380 African American cases and 253 African American controls (Ittiwut et al., 2012). They found no link between the GABRA2 and the GABRG1 polymorphisms and alcohol dependence. Although they stated certain differences between individuals concerning their personal attributes and behaviour, they could show a significant association between these polymorphisms and alcohol dependence.

Soyka et al. (2007) examined 316 patients of alcohol dependence compared with the results of a control sample of 295 non-alcoholic individuals. As laboratory procedures they used the snapshot methodology and multiplex PCR so that they could mark the existing SNPs with fluorescently labeled dideoxy nucleotides in the end. After identifying the genetic variations they applied some statistical methods such as the Student's t-test and D'value for linkage disequilibrium using THESIAS software (Tregouet et al., 2002). After all they could prove no association between the single nucleotide polymorphisms on chromosome 4 in the GABRA2 gene sequence and the development of alcohol dependence.

We collected the data given by studies that were mainly focussing on polymorphisms in the GABRA2 gene. As we already mentioned there was not such a clear association between one specific SNP and alcohol dependence given as in the case of alcohol dehydrogenase or aldehyde dehydrogenase. There were just several polymorphisms taken into consideration to play a role in the development of alcoholism. In our meta-analysis we included those homozygous single nucleotide polymorphisms that were analyzed by several studies. By dealing with homozygous allele carriers, we hoped to achieve significant results and make a clear statement about an association of the regarded SNP and alcohol dependence. All the SNPs we dealt with lay along the gene sequence encoding for the α2-subunit of the GABA receptor. This subunit is the best examined so far. The polymorphisms we included in our meta-analysis were all located on chromosome 4p, more exactly we considered SNPs at the following locations to be potentially associated with alcohol dependence: rs279858, rs279844, rs279869, rs279837, rs279871. Rs279858 represents a part of exon 5, rs279837 is part of intron 3, rs279844 is part of intron 4, rs 279869 and rs279871 are part of intron 6 of the GABRA2. We collected the numbers given for these single nucleotide polymorphisms and calculated the allele frequencies. After that we could differ between the wildtype alleles that are supposed to be much more common especially in the control group and the polymorphism, which one can find in a lower frequency in the control sample that should represent the general population in the best case.

We summed the results for the homozygous SNP carriers of these chromosomal areas up in **Table 3.1**:

(SNP*) Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
(rs58) Lappa-lainen	2005	USA	26	15	0,49	0,39	0,93	1,69 (0,79-3,68)
(rs69) Lappa-lainen	2005	USA	27	15	0,5	0,38	0,94	1,77 (0,84-3,86)

(rs37) Lappa-lainen	2005	USA	27	16	0,48	0,39	0,87	1,64 (0,79-3,52)
(rs58) Fehr	2006	Germany	50	12	0,44	0,34	0,43	1,53 (0,75-3,33)
(rs44) Fehr	2006	Germany	57	13	0,48	0,36	0,46	1,64 (0,83-3,46)
(rs37) Fehr	2006	Germany	50	11	0,45	0,34	0,76	1,68 (0,81-3,79)
(rs71) Fehr	2006	Germany	50	12	0,44	0,34	0,43	1,53 (0,75-3,33)
(rs71) Drgon (AA)	2006	USA	2	1	0,2	0,23	0,03	0,82 (0,04-49,11)
(rs71) Drgon (Cauc.)	2006	USA	4	32	0,27	0,39	0,91	0,05 (0,01-0,17)
(rs58) Soyka	2007	Germany	60	56	0,42	0,41	0,08	1,00 (0,65-1,53)
(rs44) Soyka	2007	Germany	60	42	0,43	0,39	0,55	1,41 (0,89-2,23)
(rs69) Soyka	2007	Germany	61	48	0,42	0,4	0,63	1,23 (0,79-1,91)
(rs37) Soyka	2007	Germany	55	41	0,43	0,38	0,77	1,3 (0,82-2,08)
(rs58) Covault (EA)	2008	USA	79	90	0,46	0,40	0,52	1,33 (0,94-1,89)
(rs58) Covault (AA)	2008	USA	7	9	0,24	0,24	0,07	0,5 (0,15-1,58)
(rs44) Covault	2008	USA	89	98	0,48	0,42	0,46	1,4 (1,01-1,96)

(EA)								
(rs44) Covault (AA)	2008	USA	42	28	0,47	0,48	0,05	1,02 (0,56-1,87)
(rs69) Covault (EA)	2008	USA	79	94	0,46	0,41	0,44	1,26 (0,89-1,26)
(rs69) Covault (AA)	2008	USA	7	11	0,29	0,27	0,08	0,40 (0,13-1,29)
(rs37) Covault (EA)	2008	USA	76	89	0,45	0,4	0,31	1,28 (0,90-1,83)
(rs37) Covault (AA)	2008	USA	9	10	0,26	0,27	0,13	0,58 (0,2-1,67)
(rs44) Bierut	2010	USA	118	177	0,31	0,34	<0,001	0,66 (0,51-0,84)
(rs71) Sakai (White)	2010	USA	47	16	0,47	0,42	0,39	1,92 (0,99-3,87)
(rs71) Sakai (Hisp.)	2010	USA	45	13	0,5	0,45	0,87	1,81 (0,86-4,01)
(rs69) Ittiwut	2012	USA	42	30	0,3	0,3	0,51	0,92 (0,54-1,57)
(rs37) Ittiwut	2012	USA	24	18	0,25	0,26	0,93	0,88 (0,45-1,76)

*SNP in the chromosomal location rs(2798)xx on chromosome 4p

[a]Number of cases being homozygous carrier for the polymorphism of the GABRA2

[b]Number of controls being homozygous carrier for the polymorphism of the GABRA2

[c]Minor allele frequencies for the populations' homozygous carriers of the polymorphism of the GABRA2

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association between homozygous carriers of the SNPs of the GABRA2 and alcohol dependence

Table 3.1
Characteristics of the studies dealing with the homozygous carriers for the regarded SNP given by author, year, country, number of cases and controls that are carrier of the polymorphism, minor allele frequencies, HWE (p-value) and odds ratios with 95% confidence intervals (Cauc.=Caucasian, Hisp.=Hispanic, EA=European American, AA=African American).

We had to exclude the studies by Uhart et al. (2013), Villafuerte et al. (2012) and Arias et al. (2014) because they didn't deal with control groups. Furthermore the studies by Pierucci-Lagha et al. (2005) had to be excluded because they were only examining 27 participants thus not meeting our inclusion criteria of group sizes at least of 40 participants in cases and controls. Lydall et al. (2011) and Perry et al. (2013) had to be excluded because they were not showing genotypic distributions.

Because of the fact that these SNPs of GABRA2 have all been considered to play a role in alcohol dependence, we first created a forest, in which we evaluated the risk of becoming alcohol dependent by being homozygous carrier of the SNP in comparison to heterozygous allele carriers and subjects that are homozygous for the wildtype allele and later on we compared each individual of the homozygous SNPs with other genotypes and the risk of becoming alcohol dependent.

Summary meta-analysis for homozygous carriers of GABRA2 SNPs

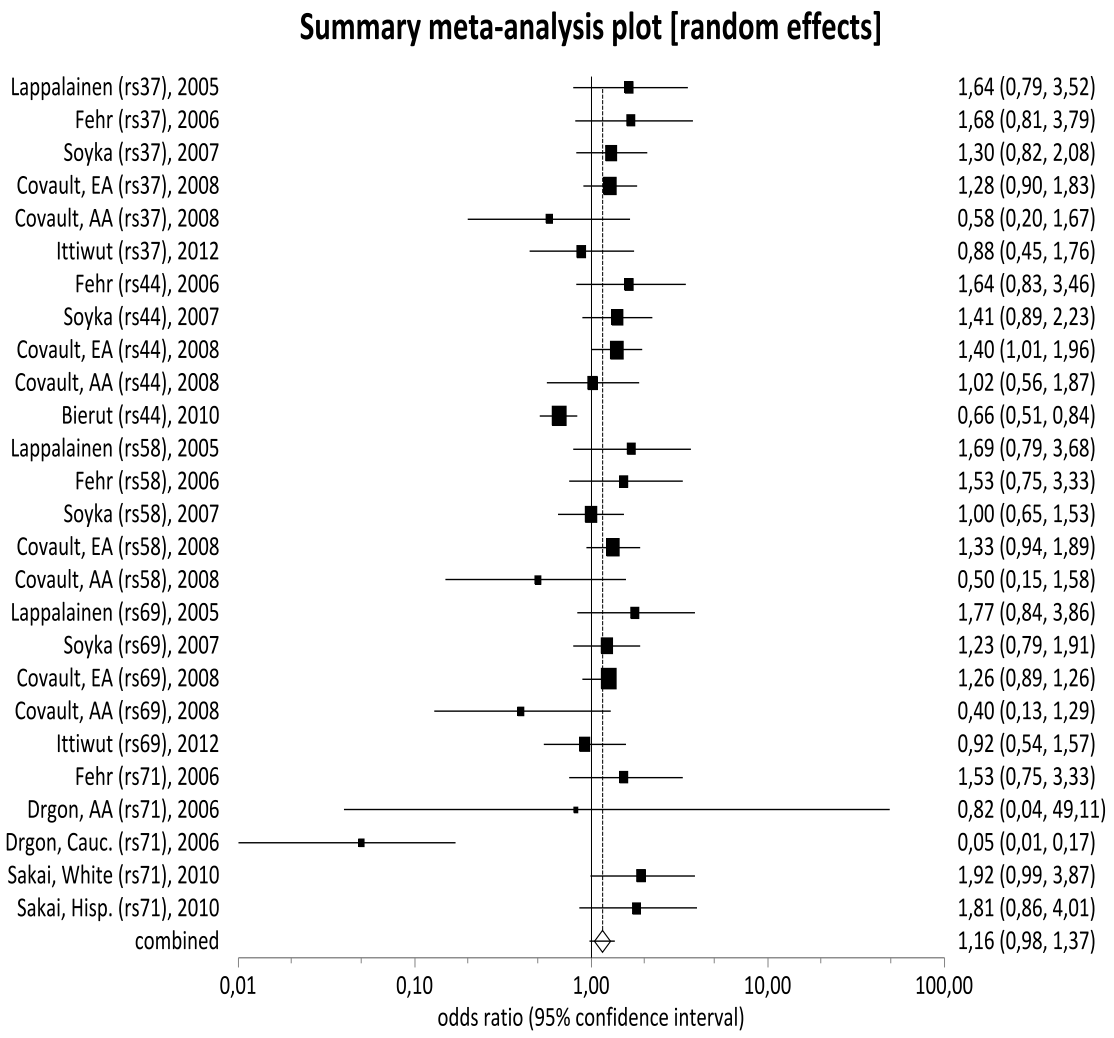


Figure 3.1
Association of the homozygous carriers of the GABRA2 SNPs with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (Hisp.=Hispanic, EA=European American, AA=African American).

Results of the GABRA2 SNPs meta-analysis: The meta-analysis implied no significantly increased or decreased risk for alcohol dependence by being homozygous carrier of one of the so far best examined SNPs in the GABRA2 (OR=1,16; 95% CI=0,98-1,37). Thus the

combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,05 to 1,92. The Cochran Q test ($P<10^{-4}$) confirmed quite high between-study heterogeneity, so did the inconsistency test (I^2=58,7%). This led to the application of a random effects model (DerSimonian-Laird).

These studies that were all dealing with Caucasians or individuals living in America (African American, European American, Hispanic) didn't report an alteration in the risk for becoming alcohol dependent by being a homozygous carrier of one of the five single nucleotide polymorphisms of the GABRA2 that have been considered to play a role in the development of alcohol dependence by some authors.

Discussion of the GABRA2 SNPs meta-analysis: The α2-subunit is supposed to be the most common one in receptors in the limbic region thus having influence on the emotional reactions after ethanol consumption that can be altered by polymorphisms in this subunit (McKernan and Whiting, 1996). We compared each of the SNPs of the GABRA2 with the heterozygous and the homozygous wildtype allele carriers of this SNP in other forest plots below. We could not use the data about the rs279871 (base exchange from thymine to cytosine) because the study by Drgon et al. (2006) didn't deliver useful data as the 95% confidence interval was too large indicating the low amount of homozygous SNP carriers in this population. So we excluded that study from the second analysis, hence having not enough results about this SNP in intron 6. We also had a look after the influence of the year the study was processed on the results of the SNPs on the risk of becoming alcohol dependent. Therefore we listed the studies in another order, from the oldest studies to the latest ones, in **Figure 5.1** in the **Attachment**.

We looked for publication bias in the funnel plot below:

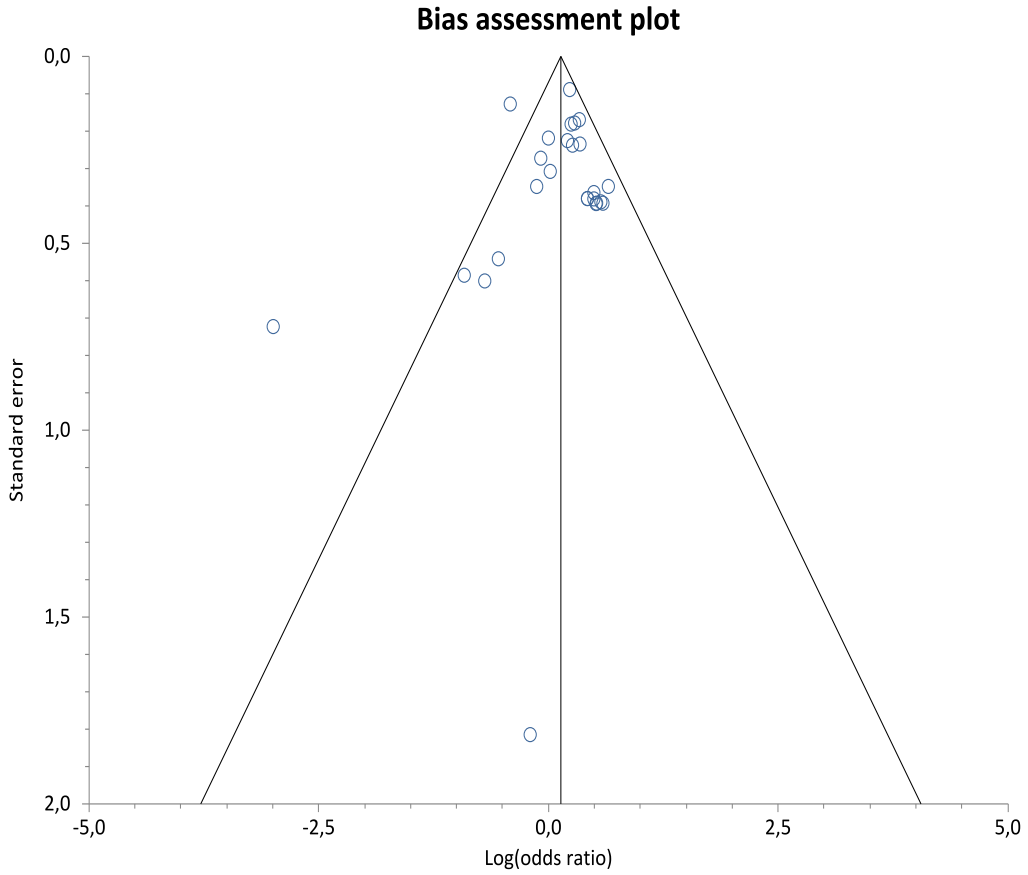


Figure 3.2
The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 polymorphisms rs279871, rs279837, rs279869, rs279844 and rs279858 with alcohol dependence. Bias indicators showed no significant deviation from the symmetry assumption and thus not indicating publication bias. Egger's regression test: P=0,71.

All these SNPs of the GABRA2 have been considered to increase the risk of becoming alcohol dependent in comparison to the wildtype allele (Covault et al., 2008). The GABA system‘s major receptor subtype, the pentameric GABA-A receptor, mediates several important effects of alcohol including sedation, anxiolysis, impairment of motor coordination and withdrawal symptoms (Buck et al., 1996) that are all associated with alcohol consumption. As polymorphisms of the α2 subunit of the GABA-A receptor alter GABAergic neurotransmission, they could possibly be associated with alcohol dependence. At least it became clear that GABA-A agonists increase, during GABA-A antagonists decrease alcohol

intake in rats (Nowak et al., 1998). We did not find an association between the homozygous carriers for all the polymorphisms together and the likeliness of becoming alcohol dependent. So we wanted to have a look on the meaning of each SNP on the likelihood of becoming an alcoholic:

Summary meta-analysis for the homozygous SNP in the GABRA2 gene, rs279837

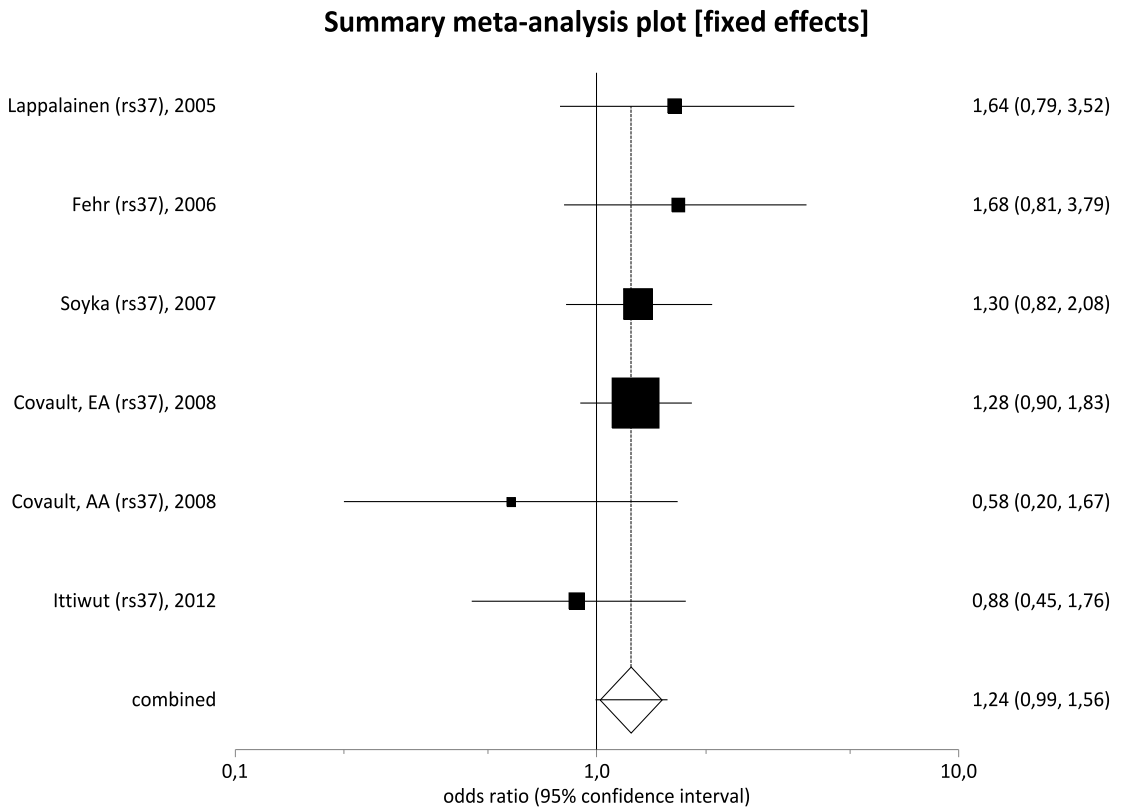


Figure 3.3
Association of the homozygous carriers of the GABRA2 SNP in rs279837 with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (EA=European American, AA=African American).

Resutls of the rs279837 meta-analysis: The meta-analysis implied no significantly increased or decreased risk for alcohol dependence by being homozygous carrier of the SNPs in the GABRA2, rs279837 (OR=1,24; 95% CI=0,99-1,56). Thus the combined effect revealed no

significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,58 to 1,68. The Cochran Q test (P=0,53) confirmed quite little between-study heterogeneity, so did the inconsistency test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel method). This SNP doesn't seem to be associated with clinically significant alterations in GABAergic neurotransmission influencing the risk of becoming alcohol dependent. As the meta-analysis suggests, the homozygous SNP in rs279837, leading to base exchange from thymine to cytosine, seems to increase the risk of becoming alcohol dependent at least slightly in Caucasians or European Americans that can ethnically be compared with Caucasians (Fehr et al., 2006; Lappalainen et al., 2005; Soyka et al., 2007; Covault (EA) et al., 2010). In contrast to that, the homozygous SNP in GABRA2, rs279837 seems to decrease the risk for alcohol dependence in African American samples (Ittiwut et al., 2012; Covault (AA) et al., 2010).

Discussion of the rs279837 meta-analysis: Rs279837 is a chromosomal region that is part of intron 3 of the α2 subunit of the pentameric GABA-A receptor (Fehr et al., 2006). As an intronic region, rs279837 cannot alter the amino acid sequence of the α2 subunit directly but it can process regulative functions that have influence on the amount of subunits created for example. Thus a polymorphism in that genetic location potentially can influence GABA transmission and reactions towards alcohol consumption. As already stated we gained opposite results for Caucasian and African American group samples. By separating these two ethnic groups, we would probably get significant associations between this polymorphism and alcohol dependence. The problem consisted in the fact, that we only found these studies dealing with the SNP in GABRA2, rs279837, hence not being able to analyze both separately in different meta-analyses. So in the end we didn't detect an alteration in the risk of becoming alcohol dependent by being homozygous carrier of the SNP in rs279837 of the GABRA2 gene compared with other genotypes of that chromosomal area.

As a sensitivity analysis we were looking for publication bias:

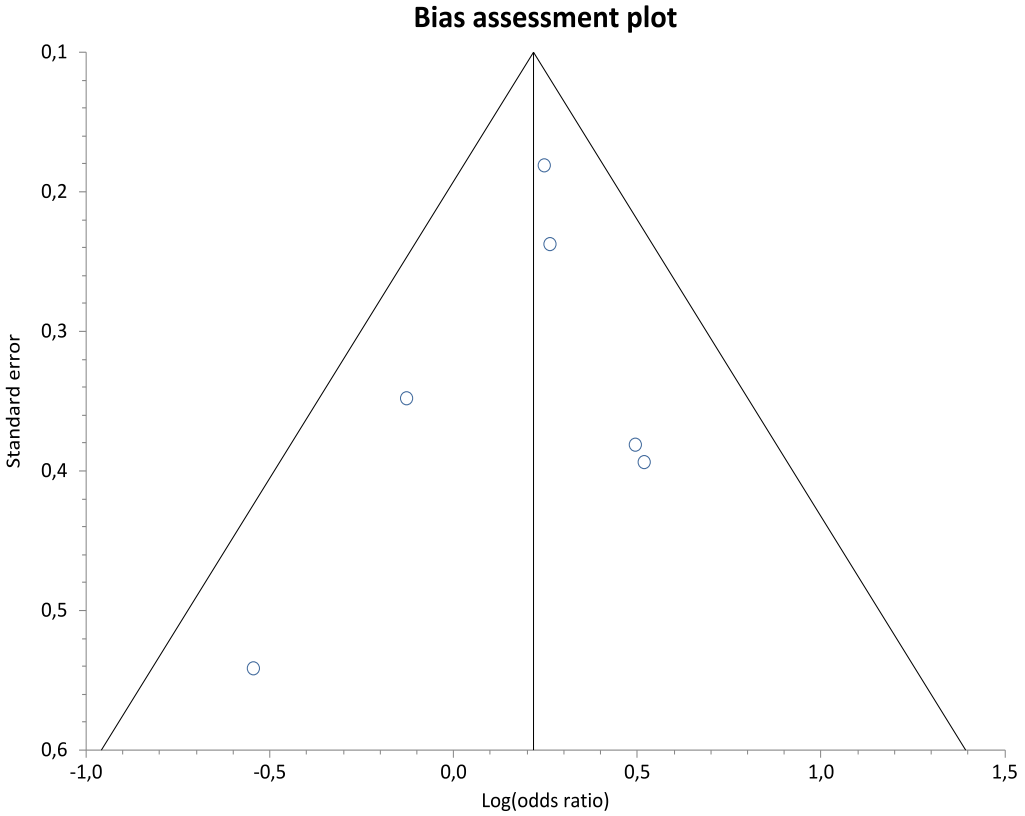


Figure 3.4
The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 (rs279837) polymorphism with alcohol dependence. Bias indicators showed no significant deviation from the symmetry assumption and thus not indicating publication bias. Egger's regression test: P=0,52.

We checked if the SNP rs279844 in the GABRA2 gene is associated with alcohol dependence, leaving out the study of Bierut et al. (2010) because their control group sample didn't meet HWE (p<0,001):

Summary meta-analysis for the homozygous SNP in the GABRA2 gene, rs279844

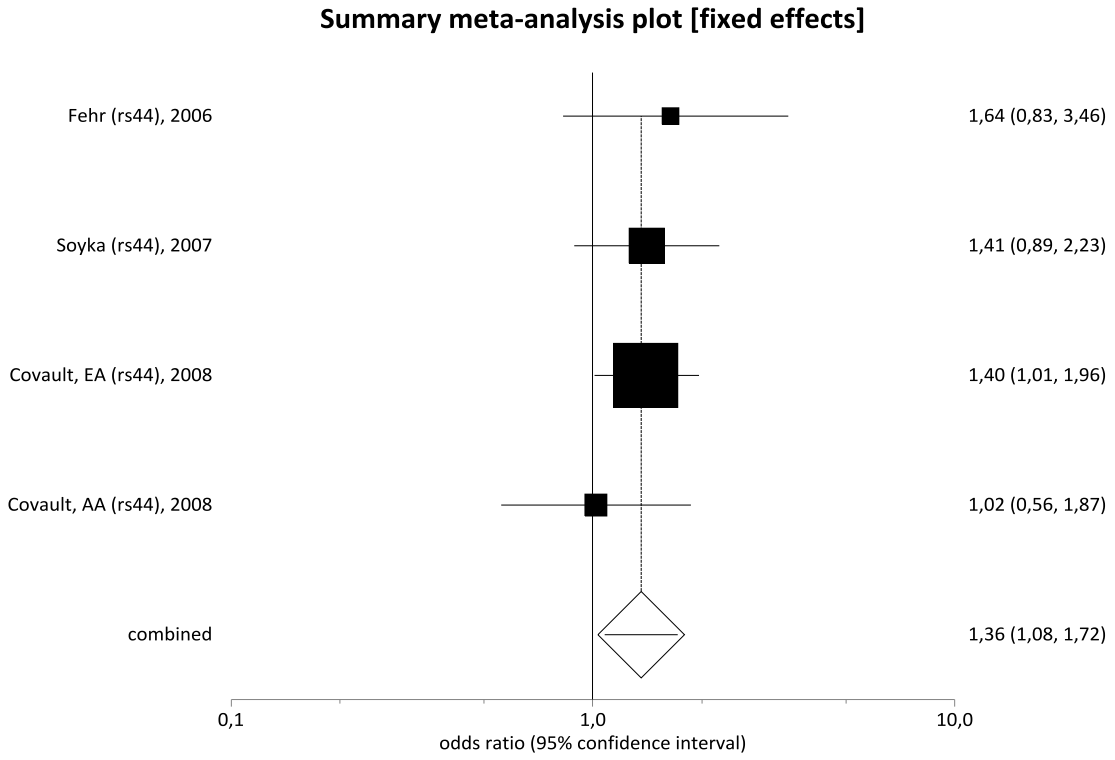


Figure 3.5
Association of the homozygous carriers of the GABRA2 SNP in rs279844 with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (EA=European American, AA=African American).

Results of the rs279844 meta-analysis: The meta-analysis implied a slightly increased risk for alcohol dependence by being homozygous carrier of the SNPs in the GABRA2, rs279844 (OR=1,36; 95% CI=1,08-1,72). Thus the combined effect revealed a significant association between the SNP and alcoholism. The individual odds ratios ranged from 1,02 to 1,64. The Cochran Q test (P=0,75) confirmed quite little between-study heterogeneity, so did the inconsistency test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel method).

The SNP rs279844 which consists in a base exchange from thymine to adenine in the GABRA2 gene is associated with a slightly increased risk for alcohol dependence. Homozygous carriers of the mutation have a 1,36 fold risk of becoming alcohol dependent compared with

heterozygous genotypes and homozygous wildtype allele carriers. The ethnic groups we are referring to are either Caucasians or European Americans and African Americans, in which the effect of the homozygous polymorphism wasn't recognisable in contrast to the studies with white participants.

Discussion of the rs279844 meta-analysis: As this chromosomal area is part of intron 4 of the α2 subunit, the regulative effect this intron has on the translation of this protein turned out be significant. The exact meaning for the transcription of the α2 subunit however remains unknown.

We were looking for publication bias in the funnel plot below:

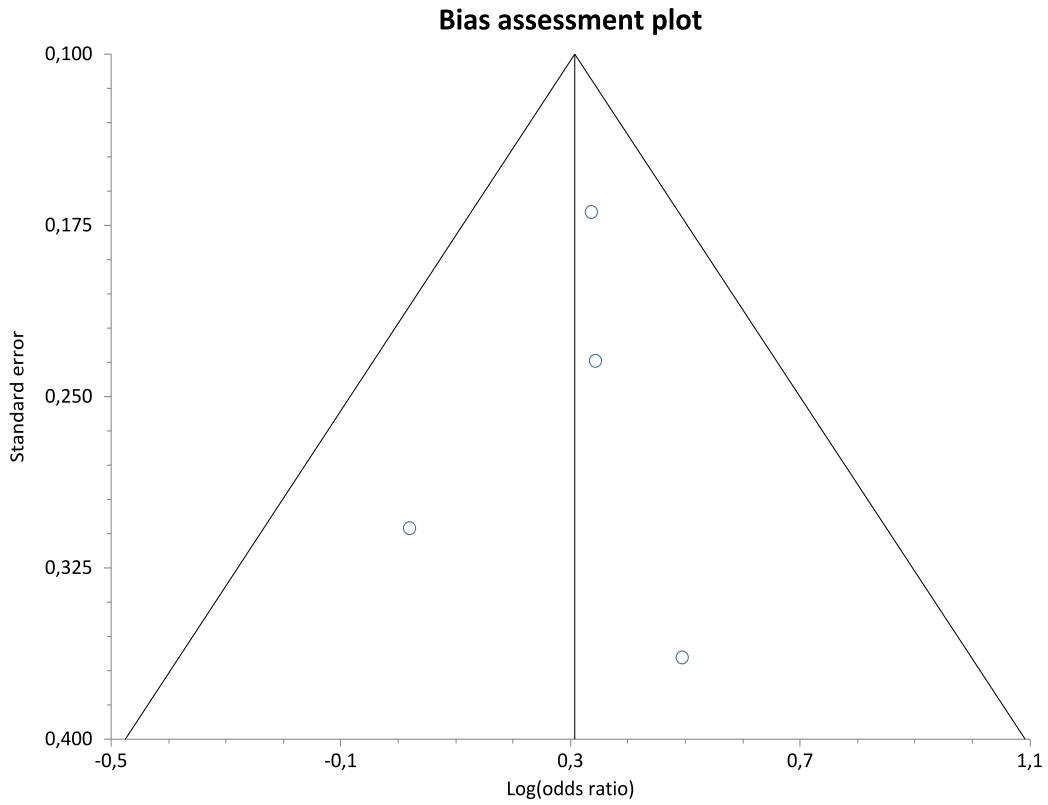


Figure 3.6
The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 (rs279844) polymorphism with alcohol dependence. Bias indicators showed no significant deviation from the symmetry assumption and thus not indicating publication bias. Egger's regression test: P=0,83.

We also clarified the role of the homozygous carriers of the SNP in GABRA2, rs279858 (base exchange from guanine to adenine), for the risk of becoming alcohol dependent:

Summary meta-analysis for the homozygous SNP in the GABRA2 gene, rs279858

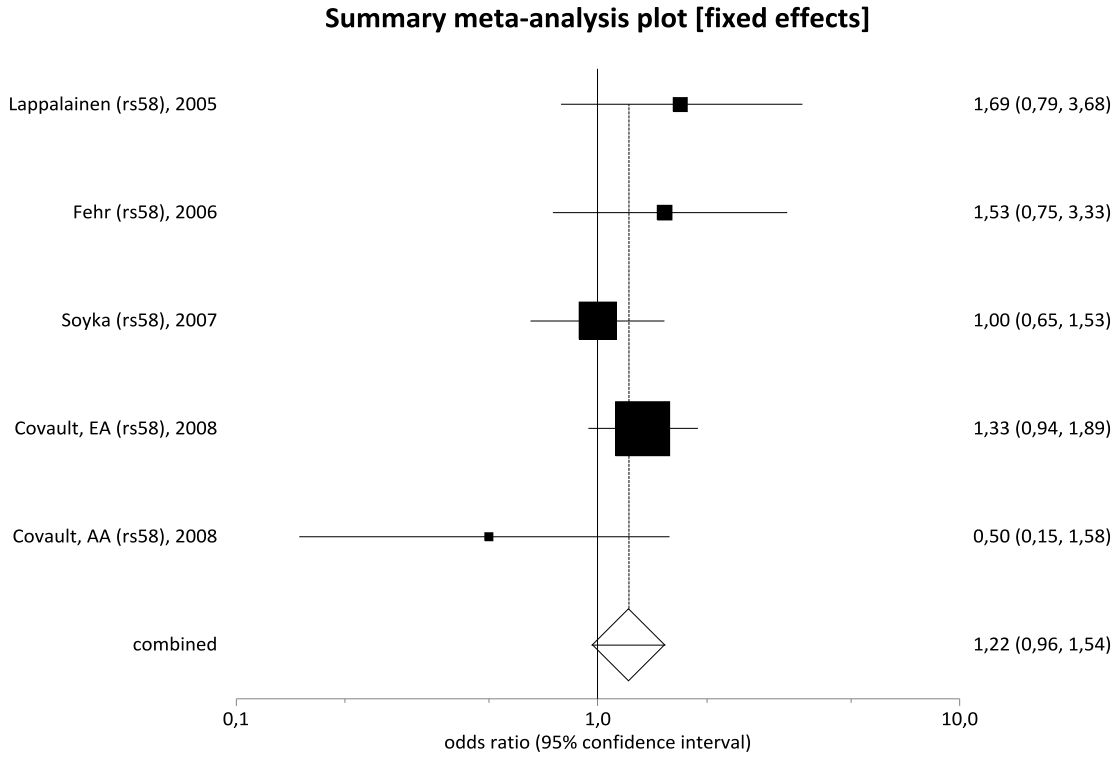


Figure 3.7
Association of the homozygous carriers of the GABRA2 SNP in rs279858 with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (EA=European American, AA=African American).

Results of the rs279858 meta-analysis: The meta-analysis implied no significantly increased or decreased risk for alcohol dependence by being homozygous carrier of the SNPs in the GABRA2, rs279858 (OR=1,22; 95% CI=0,96-1,54). Thus the combined effect revealed no significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,50 to 1,69. The Cochran Q test (P=0,37) confirmed quite little between-study

heterogeneity, so did the inconsistency test (I^2=7,2%). This led to the application of a fixed effects model (Mantel-Haenszel method).

The studies reported no significant association between alcohol dependence and the homozygous polymorphism (Lappalainen et al., 2005; Fehr et al., 2006; Covault (EA) et al., 2008). Again the African American population in the study by Covault et al. (2008) seemed to be protected by the SNP in the GABRA2 gene, although also without giving significant numbers as the 95% confidence interval is quite large due to a low number of homozygous carriers of the SNP in this chromosomal region. After all there is no association between the homozygous carriers of this SNP and alcohol dependence given in our meta-analysis.

As a sensitivity analysis we were looking for publication bias in the following funnel plot:

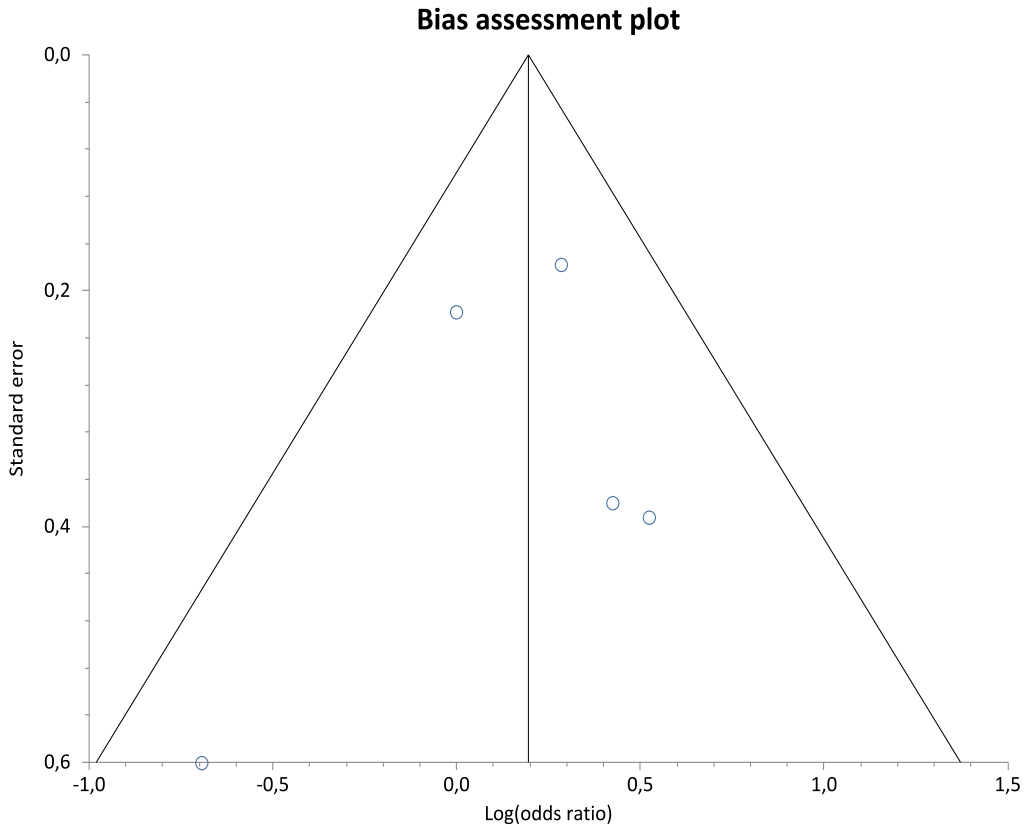


Figure 3.8

The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 (rs279858) polymorphism with alcohol dependence. Bias indicators

showed no significant deviation from the symmetry assumption and thus not indicating publication bias. Egger's regression test: P=0,71.

The last SNP in the GABRA2 gene, that we regarded to be able to cause an altered risk for becoming alcohol dependent by influencing GABAergic neurotransmission in a clinically significant way, is located at rs279869 on chromosome 4p and consists in a base exchange from cytosine to adenine. The SNPs in rs279871 (thymine to cytosine) could not be considered because the study Drgon et al. (2005) had to be excluded not meeting our criteria of having a 95% confidence interval not bigger than 8. So the meaning of this SNP in the progress of alcohol dependence could not be evaluated in an individual meta-analysis because there were not enough studies providing information about this polymorphism after excluding the one by Drgon et al. (2005).

The results from the studies dealing with the SNPs located on rs279869 are shown below:

Summary meta-analysis for the homozygous SNP in the GABRA2 gene, rs279869

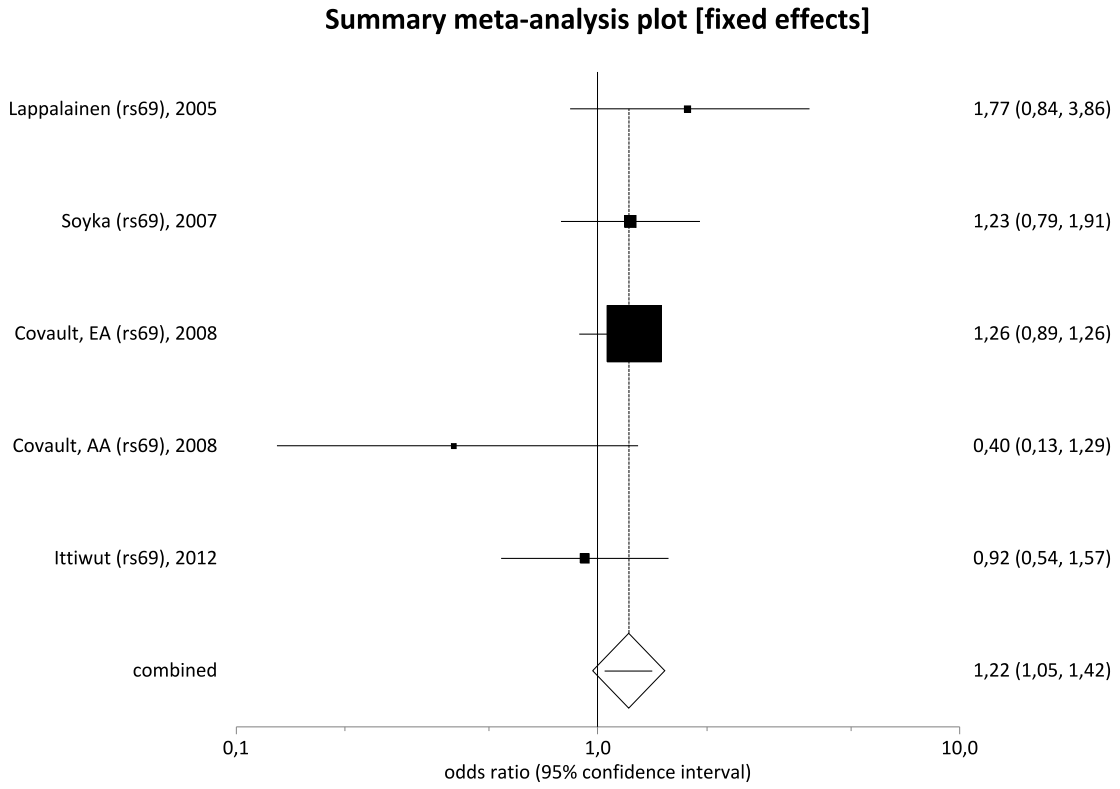


Figure 3.9
Association of the homozygous carriers of the GABRA2 SNP in rs279869 with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (EA=European American, AA=African American).

Results of teh rs279869 meta-analysis: The meta-analysis implied a slightly increased risk for alcohol dependence by being homozygous carrier of the SNPs in the GABRA2, rs279869 (OR=1,22; 95% CI=1,05-1,42). Thus the combined effect revealed a significant association between the SNP and alcoholism. The individual odds ratios ranged from 0,40 to 1,77. The Cochran Q test (P=0,22) confirmed quite little between-study heterogeneity, so did the inconsistency test (I^2=30,4%). This led to the application of a fixed effects model (Mantel-Haenszel method).

We found similar results to the analysis of the homozygous SNPs in rs279844. The European American as well as the Caucasian group samples showed a positive association with alcohol

dependence during the African American subjects were protected by the mutations. In the end there also results a bringing forward effect of the less common alleles in a homozygous stamping compared to the individuals that were heterozygous or homozygous for the wildtype allele.

Discussion of the rs279869 meta-analysis: The results though may just report a positive association because the majority of the studies we could include dealt with ethnic groups of European ancestry. If we had been able to include more studies dealing with with African Americans, we would probably achieve opposite results as the data of the studies by Ittiwut et al. (2012) and Covault et al. (2008) suggests.

We were looking for publication bias in the following funnel plot:

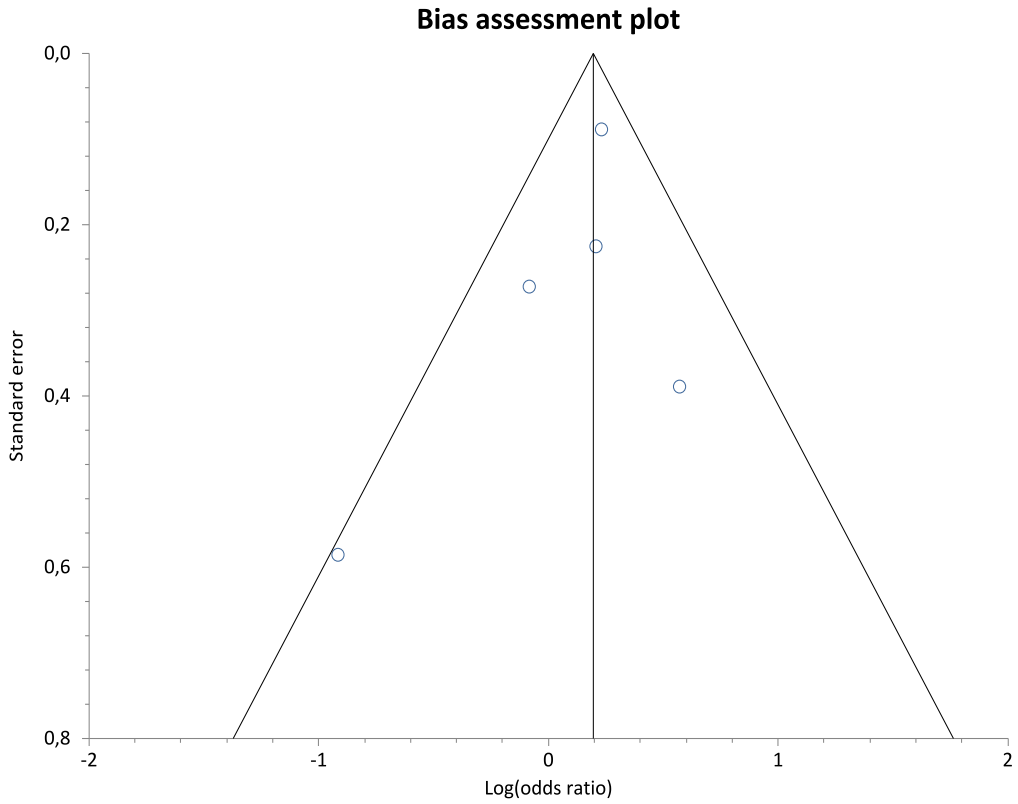


Figure 3.10
The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 (rs279869) polymorphism with alcohol dependence. Bias indicators showed no significant deviation from the symmetry assumption and thus not indicating publication bias. Egger's regression test: P=0,40.

After analyzing all the SNPs in GABRA2 for which we found several studies regarding these SNPs to be possibly linked with alcohol dependence, we cannot make a clear statement about the influence of homozygosity in these SNPs and the risk of becoming alcohol addicted. The role of GABA for the development of alcohol dependence is much more complex as the one of the alcohol dehydrogenase and the one of aldehyde dehydrogenase. While these enzymes potentially alter the risk of regular alcohol consumption by different kinetics, the role of the neurotransmitter GABA is not explained that easily. Alcohol can increase the amount of GABA in certain brain regions during it decreases the concentration of GABA in other regions at the same time (Enoch et al., 2008). The GABA-A receptors, of course, are also involved in different reactions towards alcohol, as they regulate the functioning of the neurotransmitter (Ittiwut et al., 2012). Lots of GABA-A receptors which are assembled by several subunits contain the α2-subunit which is thought to be involved in alcohol reactions in a special kind because it is the major subunit in the limbic system which is involved in the processing of addictions (Buck et al., 1996). This is why we particularly found studies that dealt with this subunit and the risk of developing alcohol dependence. The problem consisted in the fact that there was not a single SNP considered to be associated with alcohol dependence, as it was the case for the alcohol dehydrogenase and aldehyde dehydrogenase polymorphisms, but rather several polymorphisms along the GABRA2 gene that were linked with alcohol dependence and therefore compared with the different genotypes. We attended to the homozygous carriers for the relative SNP, first analyzing all the SNPs along the GABRA2 gene together (**Figure 3.1**). There was no clear association between the polymorphisms and alcohol dependence in comparison to the wildtype allele carriers. Then we were analyzing any of the regarded single nucleotide polymorphisms along the GABRA2 gene separately. We found a slightly positive association between the two SNPs rs279844 and rs279869 of the GABRA2 gene and alcoholism, in particular for European American and Caucasian subjects that are originally from the same ancestry. On the other hand we found the tendency of a negative association between these two SNPs and alcoholism in African American subjects. Therefore we suggest that there exists an inter-ethnic difference in the consequences of being homozygous carrier for one of the mutations. We did not find an association between the SNPs in rs279858 and rs279837 and alcoholism. Altogether the fact that GABAergic neurotransmission plays a role in the progress of becoming alcohol dependent is considered to be clear (Nowak et al., 1998), the

exact way isn't clarified yet. That's why we were looking for genetic variance that influences GABA reception, especially in the α2-subunit which is supposed to be involved in the development of addictions in a specific manner (Buck et al., 1996). We can't be sure how big the impact of genetically altered GABA-A receptor functioning is on the overall risk of developing alcoholism but we found quite good evidence that an influence exists. Still it will be necessary to do further research to clarifiy which SNP is responsible for the alteration in the risk of becoming alcohol dependent and which SNP among the GABRA2 gene is not involved in the complex pathway of genetic predisposing towards alcohol dependence.

We could also consider the homozygous carriers of the more common alleles of the GABRA2 gene, the wildtype alleles, to be either protected from alcohol dependence or vulnerable towards regular alcohol consumption. That's why we investigated a meta-analysis dealing with the homozygous carriers of the wildtype alleles of the GABRA2 gene in comparison to the carriers of the polymorphisms, either in homozygous or heterozygous occurrence.

Therefore we summed the data given by the studies included up in **Table 3.2**:

(locus*) Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
(rs58) Lappa-lainen	2005	USA	28	37	0,49	0,39	0,93	0,56 (0,29-1,05)
(rs69) Lappa-lainen	2005	USA	27	38	0,5	0,38	0,94	0,51 (0,27-0,96)
(rs37) Lappa-lainen	2005	USA	31	37	0,48	0,39	0,87	0,64 (0,34-1,19)
(rs58) Fehr	2006	Germany	78	39	0,44	0,34	0,43	0,54 (0,32-0,93)
(rs44) Fehr	2006	Germany	69	37	0,48	0,36	0,46	0,51 (0,29-8,67)

(rs37) Fehr	2006	Germany	74	38	0,45	0,34	0,76	0,53 (0,31-0,91)
(rs71) Fehr	2006	Germany	78	39	0,44	0,34	0,43	0,54 (0,32-0,93)
(rs71) Drgon (AA)	2006	USA	136	52	0,2	0,23	0,03	1,2 (0,71-2,02)
(rs71) Drgon (Cauc.)	2006	USA	108	76	0,27	0,39	0,91	0,69 (0,47-1,01)
(rs58) Soyka	2007	Germany	111	111	0,42	0,41	0,08	0,89 (0,64-1,26)
(rs44) Soyka	2007	Germany	104	108	0,43	0,39	0,55	0,85 (0,6-1,2)
(rs69) Soyka	2007	Germany	111	110	0,42	0,4	0,63	0,91 (0,64-1,28)
(rs37) Soyka	2007	Germany	109	113	0,43	0,38	0,77	0,85 (0,6-1,19)
(rs58) Covault (EA)	2008	USA	110	195	0,46	0,40	0,52	0,73 (0,54-1,05)
(rs58) Covault (AA)	2008	USA	85	61	0,24	0,24	0,07	0,86 (0,49-1,49)
(rs44) Covault (EA)	2008	USA	107	182	0,48	0,42	0,46	0,78 (0,58-1,05)
(rs44) Covault (AA)	2008	USA	34	32	0,47	0,48	0,05	0,63 (0,34-1,16)
(rs69) Covault (EA)	2008	USA	106	191	0,46	0,41	0,44	0,72 (0,53-0,96)

(rs69) Covault (AA)	2008	USA	69	56	0,29	0,27	0,08	0,68 (0,39-1,18)
(rs37) Covault (EA)	2008	USA	112	196	0,45	0,4	0,31	0,74 (0,55-0,99)
(rs37) Covault (AA)	2008	USA	79	57	0,26	0,27	0,13	0,86 (0,50-1,48)
(rs44) Bierut	2010	USA	842	813	0,31	0,34	<0,001	1,09 (0,96-1,25)
(rs71) Sakai (White)	2010	USA	58	32	0,47	0,42	0,39	1,05 (0,61-1,85)
(rs71) Sakai (Hisp.)	2010	USA	45	20	0,5	0,45	0,87	1,02 (0,52-2,04)
(rs69) Ittiwut	2012	USA	193	129	0,3	0,3	0,51	0,99 (0,71-1,38)
(rs37) Ittiwut	2012	USA	211	137	0,25	0,26	0,93	1,05 (0,76-1,47)

*location on chromosome 4p, rs(2798)xx

[a]Number of cases being homozygous carrier for the wildtype allele of the GABRA2

[b]Number of controls being homozygous carrier for the wildtype allele of the GABRA2

[c]Minor allele frequencies for the populations' homozygous carriers of the wildtype allele of the GABRA2

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association between homozygous carriers of the witldtype allele of the GABRA2 and alcohol dependence

Table 3.2
Characteristics of the studies dealing with the homozygous carriers for the regarded wildtype alleles given by author, year, country, number of cases and controls that are carrier of the polymorphism, minor allele frequencies, HWE and odds ratios with confidence intervals (Cauc.=Caucasian, Hisp.=Hispanic, EA=European American, AA=African American).

We had to exclude the studies by Uhart et al. (2013), Villafuerte et al. (2012) and Arias et al. (2014) because they didn't deal with control groups. Furthermore the studies by Pierucci-Lagha et al. (2005) had to be exluded because they were only examining 27 participants thus not meeting our inclusion criteria of group sizes at least of 40 participants in cases and controls. Lydall et al. (2011) and Perry et al. (2013) had to be excluded because they were not showing genotype distributions.

We compared the risk of becoming alcohol dependent for homozygous genotypes of the regarded wildtype alleles of the GABRA2 gene in comparison to heterozygous genotypes and genotypes that are homozygous for the single nucleotide polymorphism of the relative chromosomal region in the following forest plot:

Summary meta-analysis for homozygous carriers of GABRA2 wildtype alleles (1)

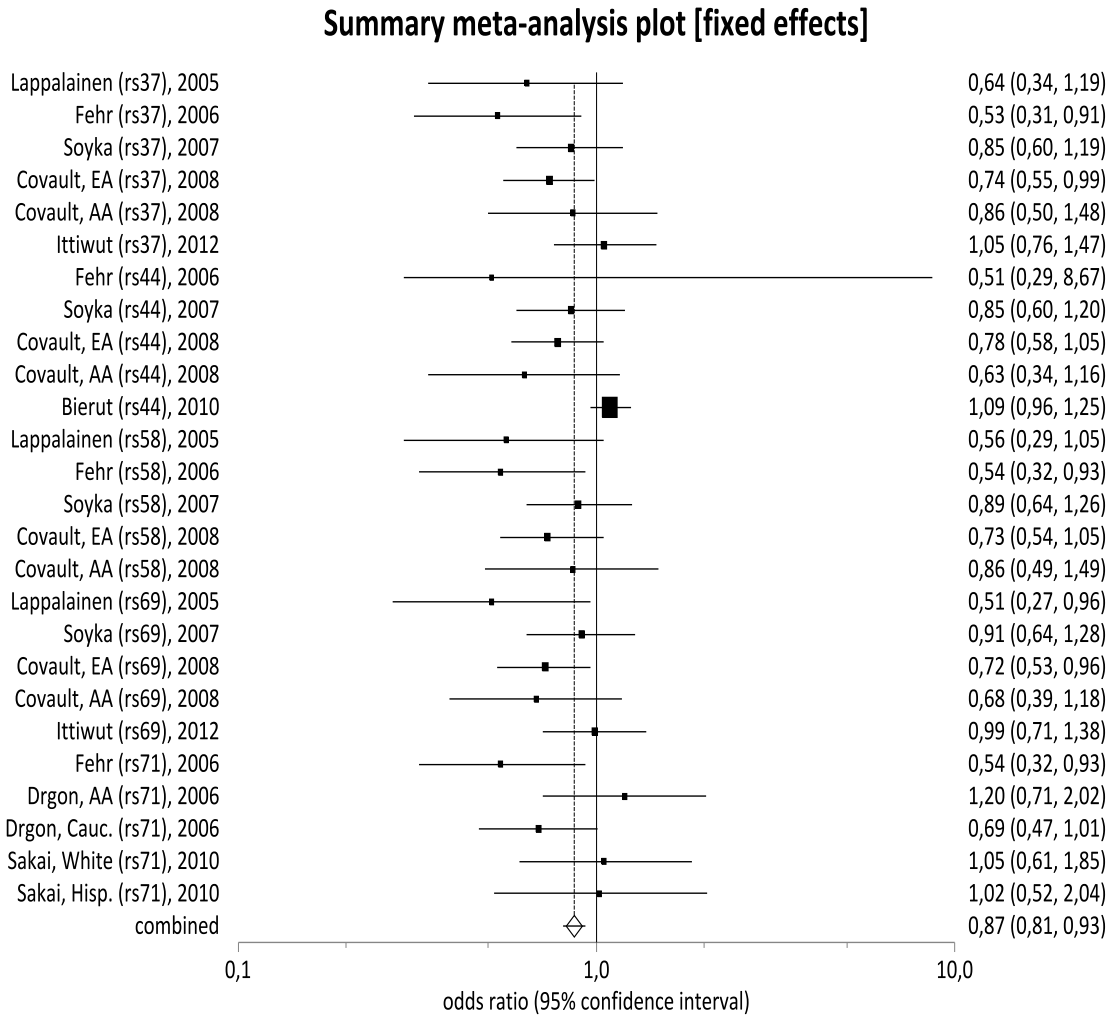


Figure 3.11
Association of the homozygous carriers of the GABRA2 wildtype alleles with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (Hisp.=Hispanic, EA=European American, AA=African American).

Results of the GABRA2 wildtype allele (1) meta-analysis: The meta-analysis implied a significantly decreased risk for alcohol dependence by being homozygous carrier of the wildtype alleles in the GABRA2 gene (OR=0,87; 95% CI=0,81-0,93). Thus the combined effect revealed a significant, negative association between this allelic constellation and alcoholism.

The individual odds ratios ranged from 0,51 to 1,20. The Cochran Q test (P=0,05) confirmed quite low between-study heterogeneity, so did the inconsistency test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel method).

Throughout nearly all the studies there was a protective effect of being homozygous carrier of the wildtype alleles in comparison to other genotypes for the development of alcohol dependence.

We were looking for publication bias in the following funnel plot:

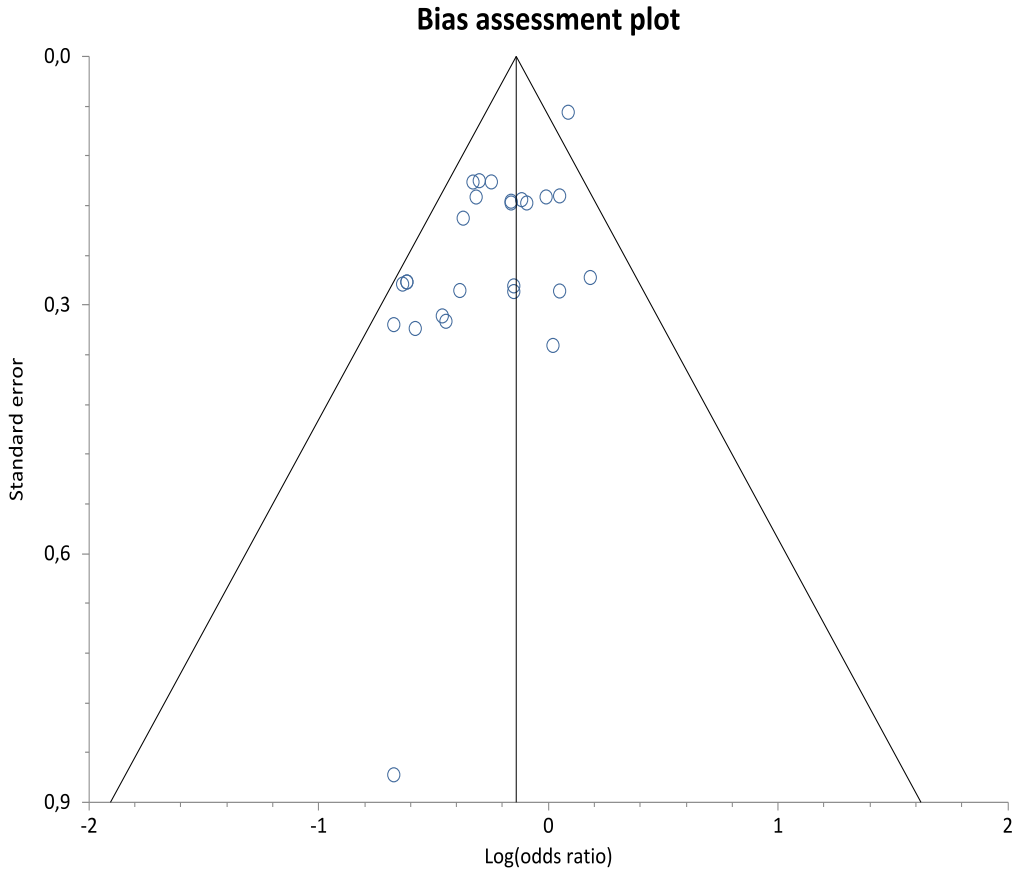


Figure 3.12

The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 wildtype allele carriers for rs279871, rs279837, rs279869, rs279844 and rs279858 with alcohol dependence. Bias indicators showed some significant deviation

from the symmetry assumption and thus indicating publication bias. Egger's regression test: P<0,001.

The results of the studies do not lie in statistical spread because there is one study that has a big weight for the bias assessment plot, which is reporting results that are controversy to the ones of the other studies (Bierut et al., 2010). As we worked through the studies, we only found study designs that we considered to be good designs fulfilling our inclusion criteria, such as case-control studies with matched-pairs techniques to collect a control group sample using structured or semi-structured questionnaires to differ between alcoholics and controls and numbers of genotypes shown as well as controlled and longitudinal cohort studies with a high compliance of the participants (Fehr et al., 2006; Sakai et al., 2010; Ittiwut et al., 2012; Covault et al., 2008; Soyka et al., 2007; Bierut et al., 2010). There was no study not meeting our inclusion criteria.

However as a sensitivity analysis we filtered out the studies that were not meeting HWE in the control group, thus not showing usual genotype distribution. There were two studies that didn’t meet HWE in the control group sample (Bierut et al., 2010; Drgon (AA) et al., 2005). Exactly these two studies were reporting another association of the homozygous wildtype allele carriers and alcoholism in comparison to all the other studies that reported a negative association or no association between the genotype and alcohol dependence. Furthermore these two studies are consequently causing publication bias having a big weight in the funnel plot, in particular this is the case with the study by Bierut et al. (2010).

So we excluded them from a second meta-analysis, hence processing our quality assessment of including studies that met HWE in the control group only. Thus we hoped to get rid of other factors influencing the studies outcome apart from genotypic distributions. Moreover we excluded studies that had 95% confidence intervals larger than 8:

Summary meta-analysis for homozygous carriers of GABRA2 wildtype alleles (2)

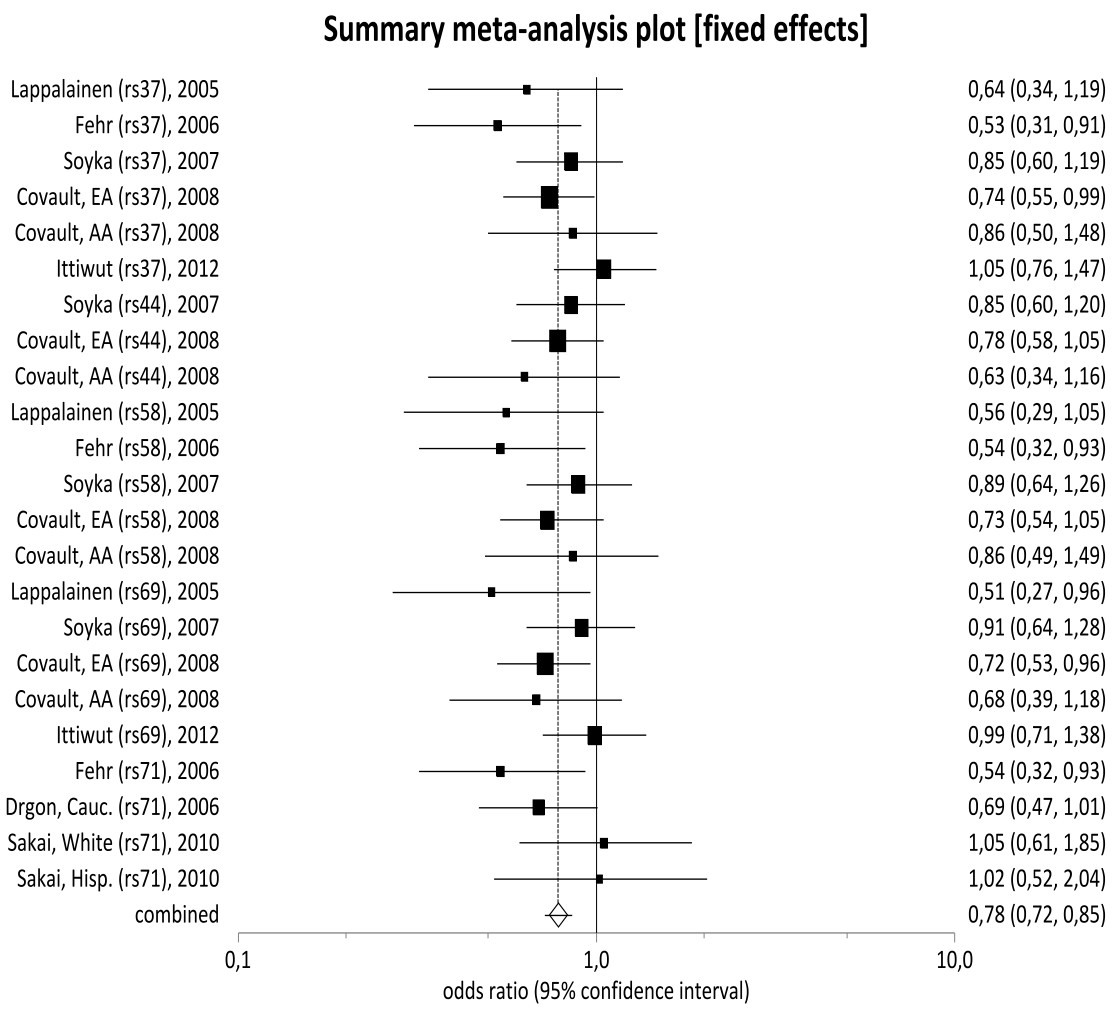


Figure 3.13
Association of the homozygous carriers of the GABRA2 wildtype alleles with alcohol dependence after excluding studies without the control samples meeting HWE and studies with 95% CIs larger than 8. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (Hisp.=Hispanic, EA=European American, AA=African American).

Results of the GABRA2 wildtype allele (2) meta-analysis: The meta-analysis implied a significantly decreased risk for alcohol dependence by being homozygous carrier of the wildtype alleles in the GABRA2 gene (OR=0,78; 95% CI=0,72-0,85). Thus the combined effect revealed a significant, negative association between this allelic constellation and alcoholism.

The individual odds ratios ranged from 0,51 to 1,05. The Cochran Q test (P=0,63) confirmed quite low between-study heterogeneity as well as the inconsistency test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel method).

What we gained for the polymorphisms was a positive association between the homozygous carriers of the SNPs in rs279844 and rs279869 and no association between the homozygous carriers of the SNPs in rs279837 and rs279858 with alcohol dependence. Moreover there was a clear difference between ethnicities of white or European ancestry and African American subjects. For the homozygous wildtype allele carriers there is no difference between ethnicities reported. Regardless from the ethnic background of the analyzed group there was either a negative association between being homozygous carrier for the wildtype allele and alcohol dependence or no association between this genotype and the addiction (Ittiwut et al., 2012; Sakai et al., 2010).

Discussion of the GABRA2 wildtype allele (2) meta-analysis: After excluding several studies from our second meta-analysis, we still consider the homozygous wildtype allele carriers of the GABRA2 gene polymorphisms to be protected from alcoholism in comparison to other genotypes. Thus we can assume a bringing forward effect of the SNPs towards alcoholism although the homozygous genotypes of these single nucleotide polymorphisms didn't all show significant association with alcohol dependence (**Figure 3.1**). Probably being heterozygous carrier for a SNP in the GABRA2 gene is already enough to increase the risk for alcohol dependence significantly which would outline that the polymorphisms represent dominant alleles. In the end there seems to exist a link between the polymorphisms in the GABRA2 gene and alcohol dependence as homozygosity of the wildtype allele has a significantly protective impact. So the link between the GABRA2 gene polymorphisms and alcoholism exists, while the clinical relevance of each of the polymorphisms among the GABRA2 is not clarified yet. Some might be significantly associated with alcohol dependence during others are not. It will be subject of further research to evaluate the difference in the individual SNPs' meaning for alcoholism. What is more the heterozygous genotype may already be enough to end up in a higher risk for alcohol dependence compared with the homozygous wildtype allele carriers emphasizing again the fact the polymorphisms are supposed to be the dominant alleles. Probably the heterozygosity of this gene leads already to a clinically significant alteration in GABAergic transmission that alters the altogether risk

for becoming alcohol dependent and some homozygous genotypes therefore do not show any association with alcohol dependence in comparison with the other allelic combinations of the GABRA2 gene. We also had a look after the influence of the year the study was processed on the influence of the SNPs on the risk of becoming alcohol dependent. Therefore we listed the studies in another order in **Figure 5.2** in the **Attachment**.

As a sensitivity test we were looking for publication bias in the funnel plot below:

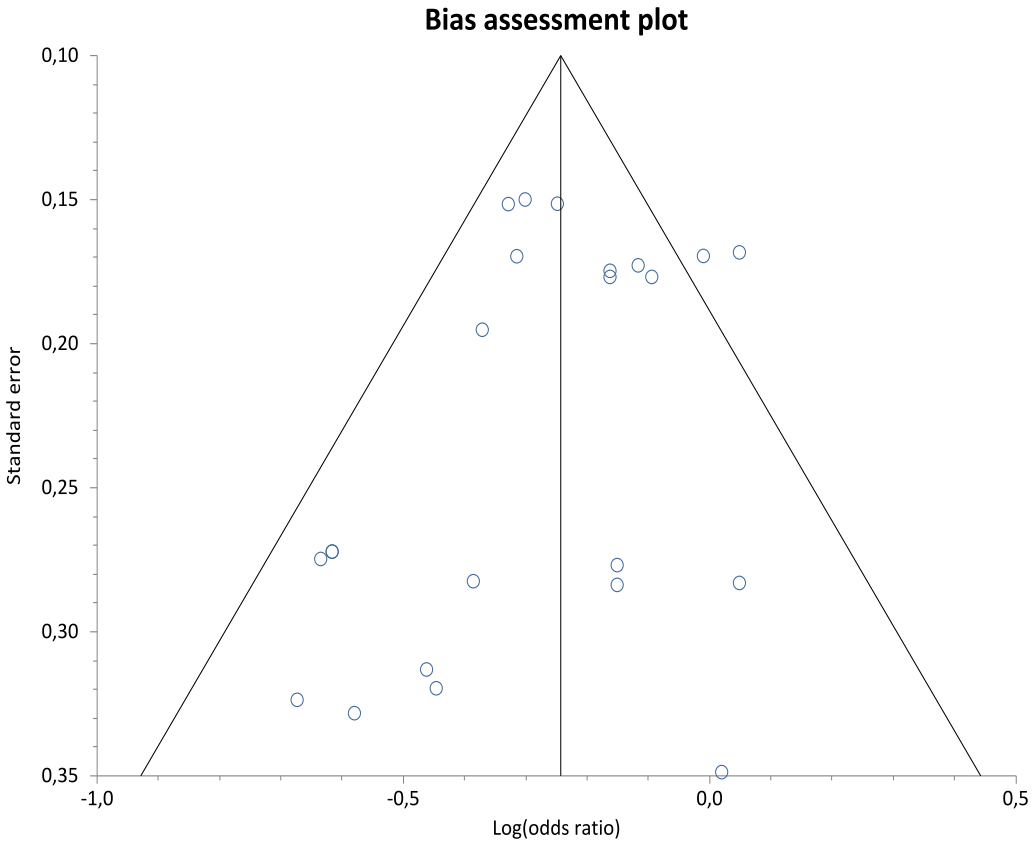


Figure 3.14
The funnel plot is showing log(OR) and standard errors for the association of the homozygous GABRA2 wildtype allele carriers for rs279871, rs279837, rs279869, rs279844 and rs279858 with alcohol dependence. Bias indicators showed no significant deviation from

the symmetry assumption and thus not indicating publication bias. Egger's regression test: P=0,06.

After we excluded the results of the studies with a 95% CI larger than 8 and the studies without their control group meeting Hardy-Weinberg-Equilibrium (p<0,05) we got rid of the publication bias. So in the end the remaining results lie in statistical spread and therefore we can consider the data to be quite reliable and valide. The publication bias in **Figure 3.12** consequently were caused by the results of the studies that admittedly processed reasonable study designs but were not meeting HWE in the control sample which stands for another factor apart from genotypic distribution blurring the studies' outcome.

Another important mutation leading to a modified subunit of a GABA receptor is also located on chromosome 4 and affects the β1-subunit. So the polymorphism of the GABRB1 has also been taken into consideration affecting the results of ethanol intake. A mouse model based analysis discovered a heritable preference for ethanol consumption with a dominant mutation in the GABRB1 gene that causes spontaneous openings of the ion channel in the nucleus accumbens that is closely associated with an alcohol addictive disease (Anstee et al., 2013). Other studies noted a weak but consistent linkage disequilibrium between GABRB1 variations and alcoholism (Song et al., 2003). They analyzed the genes on chromosome 5 encoding subunits for GABA receptors as not associated with alcohol dependence. In contrast to that GABRA5 and GABRB3 alleles on chromosome 15 provided evidence for an association in a Caucasian population, in the case of a GABRA5 mutation with good significance (p<0,004) and in the one of GABRB3 as well (p<0,007). These results were only achieved for paternal and not for maternal transmission. Even microsatellite polymorphisms have been taken into consideration when examining the effects on alcoholism and there are associations reported for the alleles of GABRB1 (Parsian et al., 1999) but no association has been found for GABRA1 and GABRA3 genes (Parsian et al., 1997). A checkup of 349 German

alcoholics and 182 ethnically matched controls attempted to give evidence for an association of SNPs in GABRA6, GABRB2 and GABRG2 alleles and alcoholism (Fehr et al., 2006). They formed three subgroups containing patients with a history of alcohol withdrawal (n=106), patients with a history of parental alcoholism (n=120) and comorbid people of dissocial personality disorder (n=57). There was no evidence given that any of the allelic variants has an effect on the development of an AD or on alcohol withdrawal symptoms of heritable alcohol addiction ($p>0,05$).

Other quantitative analyses with mice found out that the segments providing the genetic information for GABA receptor subunits such as α1, α6, β2 or γ2 lead to increased vulnerability for alcohol withdrawal severity (Enoch et al., 2008). A different experiment with mice used ethanol extract of Cirsium japonicum which stimulated GABA receptors in human neuroplastoma cells. This stimulation leads to an increase Cl^--influx that causes effects like anxiolysis and could be blocked with GABA antagonists that definitively suggested a GABA receptor channel mechanism of action (dela Pena et al., 2013). Furthermore a case-control study of 186 males with alcohol dependence and reported criminal behaviour and a group of 139 persons without previous criminal records highlighted the differences in the genotype for a SNP in rs3780428, an intronic region of GABRB2 (Terranova et al., 2013). This emphasizes the complexity of interaction between organic, psychological and behavioural effects of ethanol and its genetics. A SNP in a chromosomal region predisposing for the development of alcoholism can create several phenotypes that are not all characterized by the same pattern of behaviour. The effects of a predisposition can differ immensely but they are all together leading to an increased likeliness of becoming an alcoholic. So the meaning and mechanisms of GABA receptor mutations on alcohol dependence are much more complex than those of the ethanol disintegrating enzymes.

Some research focussed on the meaning of the exact locus in the human brain when it comes to symptoms of ethanol intake. Certain differences cause the characteristic symptoms of areas improving their neuronal information transfer and other areas regulating down their synaptic activity and action potential related work. The anterior ventral tegmental area for example was examined to have influence on the consumption of ethanol as well as on the consumption of carbon hydrates. An injection of Picrotoxin, a potent GABA antagonist, decreased ethanol intake significantly for about 55-84%, in contrast the saccharin

consumption was not altered (Nowak et al., 1998). These results link the amount of consumed alcohol with the function of the anterior ventral tegmental area and give the possibility of an intervention when it comes to alcohol abuse. In addition to that the central nucleus of the amygdala (CeA) has been connected with the effects of ethanol and other drugs. Ethanol is responsible for the activation of the corticotropin-releasing factor type 1-neurons (CRF1-neurons) that are mainly located in the medial portion of the amygdala (Herman et al., 2013). That consequently leads to the increase of CRH in the hypothalamus which is affecting the pituitary. This endocrinal gland secretes ACTH that influences the zona fasciculata at the adrenals. The result consists in a higher cortisol level with its short-term benefits for the organism such as increasing the glucose concentration in the blood or protecting the organism from subjectively aversive effects of an activated immune system. So in the end alcohol manipulates the closed loop hypothalamus-pituitary-adrenals. In the central nucleus of the amygdala there exists another cell type next to the CRF1-neurons, the so called unlabeled CeA-neurons. In mice experiments both cell types exhibited a GABA receptor subunit mediated tonic conductance driven by action potential dependent GABA release. Ethanol influenced these mechanisms by increasing the firing discharge of CRF1-neurons and decreasing the one of the unlabeled CeA-neurons (Herman et al., 2013). While other studies focussed on phasic inhibition through GABA release (Roberto et al., 2003) the latest results showed the impact of alcohol on the tonic inhibition through the GABA system that has got massive effects on other regulating other brain regions. Summarizing the experiment, it is fundamental to differ between the two cell types in the central nucleus of the amygdala that are influenced by alcohol in opposite ways. While the unlabeled cells lower their activity through ethanol, the CRF1-neurons increase their firing discharge and finally rise the cortisol level that initially rewards the organism for consuming alcohol and clears the way for more long- and short-term ethanol intake.

Regarding the effects of alcohol on the cellular level of the GABA pathway in the human brain, several studies investigated the importance of the protein kinase A (PKA) and protein kinase C (PKC). The fact that the PKC and also the PKA have influence on the GABA receptor activity (Ives et al., 2002) was examined with rats by methods as Western Blot or Patch clamp recordings. The data revealed that ethanol exposure increases membrane associated PKA and PKC activity. These two enzymes have different outcome for the GABA transmission as the PKA increases the α1-subunit surface expression by cAMP while the PKC pathway lowers

the expression of this GABA receptor subunit. These opposing effects of ethanol follow a time-dependent regulation: while the PKA activity lowers down to the baseline after 4 hours, the PKC is still active which leads to a long-term decrease of α1-subunit expression (Carlson et al., 2013). The alteration in the GABA signalling now is associated with dependence and withdrawal symptoms. This is why these enzymes represent targets for an alcohol dependence treatment. Furthermore there is a difference in neuronal action between acute ethanol intoxication and chronic ethanol intake described (Kumar and Singh, 2009). While the acute ethanol consumption increases the overall GABAergic transmission, the chronic disease entails deficits in GABA functioning. Supporting these results, other studies discovered a decrease in subunit α1 of the GABA receptor in the cerebral cortex (Devaud et al., 1997) and hippocampus (Liang et al., 2007) that as we know from the experiment mentioned above is caused by the activity alteration of PKA and PKC. To underline the significance of this subunit according to the alcohol impact, another experiment using mice with a genetic deletion of the α1-subunit showed that the tremor as a symptom of withdrawal was much more distinctive (Kralic et al., 2005). In addition to that to stress the major importance of the PKA for the ethanol effects they used a protein kinase A inhibitor like Rp-cAMP together with ethanol to decrease the GABA α1-subunit membrane levels. They consisted in a decrease by 33% which again highlights the PKA pathway leading to an increase in α1-subunit surface expression (Carlson et al., 2013).

Although some studies reported an association of other SNPs than the ones of α2-subunit with alcohol dependence, there has not been enough data to create a meta-analysis for those mutations. The reason for that might be caused by the complexity of ethanol effects on different subunits of GABA-A receptors that is quite difficult to analyse and separate from other influences on the metabolism of that neurotransmitter and its information handed by its receptors. In general, alcohol leads to an increased permeability of the channels for GABA and thus creates effects such as lower excitability for example (Devaud et al., 1997) but how the diverse types of subunits influence that fact is not explained completely, so that there is further research necessary to clarify these effects on the transmission of the major inhibitory neurotransmitter in the human brain. Furthermore there are several gauzes where the receptors are expressed, so the effects of activation are dependent on the locus we are focussing. The data of the studies that reported an association between alcoholism and

GABA-A receptor polymorphisms different from GABRA2 polymorphisms is shown in **Table 5.1**.

That leaves us with the GABRA2 receptor to compare different studies analysing the meaning of that mutations for the likeliness of becoming an alcoholic. The reason why this receptor has been identified to be associated with that disease in contrast to the SNPs in regions encoding for other subunits may lie in the fact that it is expressed in a high amount in the nucleus accumbens and in the limbic system (Enoch et al., 2008) that influences emotions which have been linked with addictions for a very long time and therefore is very nearby considered being responsible for alcohol effects and withdrawal. Moreover this subunit has already been used as a target for medication, for example benzodiazepines, so it has already been of clinical interest before it was associated with the development of alcohol dependence. What is more as we already suggested it is not quite clear yet if the influence on the likelihood of becoming alcohol dependent of the polymorphisms of the GABRA2 gene is just clinically imoportant when being homozygous carrier of the SNP or if the heterozygous genotype already alters the risk in a significant manner. This was the result of our meta-analysis representing homozygosity for the wildtype allele as a protective genotypic constellation while the influence homozygosity of the four examined SNPs only showed either a slightly increased risk for alcohol dependence or even no alteration in the risk for becoming alcohol dependent at all leaving us with the suggestion that the single nucleotide polymorphisms of the GABRA2 gene may be of dominant inheritage.

3.4) Dopamine receptor D2 genes

Dopamine is a catecholamine which is synthesized in the human brain and in vegetal nervous system in sympathetic ganglions. It derives from the essential amino acid phenylalanine with the intermediate steps tyrosine produced by the phenylalanine hydroxylase and DOPA produced by the tyrosine hydroxylase. Afterwards the decarboxylation of DOPA by the DOPA decarboxylase leads to the product dopamine (Eisenhofer et al., 2003). Dopamine has a big importance in several neuronal activities. The probably most famous one is the Parkinson's disease which is caused by a lack of dopamine in the substantia nigra that is coloured by the high content of melanine and iron. Through the lack of dopamine the disinhibition imparted by the lack of dopamine D2-receptor activation in the basal ganglion bow leads to the symptoms of the Parkinson's disease (Kaufmann et al., 2004). So the dopamine regulation became subject of a large scale of pharmaceuticals. Moreover dopamine is involved in the development of anxiety and related disorders. Thus medicaments blocking or interacting with dopamine receptors are usual therapy strategies of these psychiatric disorders. Other drugs being blockers for dopamine receptors operate as antiemetics in the area postrema (Tripanichkul et al., 2003).

Most frequently the aim of these pharmaceuticals is the dopamine receptor D2 (DRD2). This receptor is also located in a high closeness in the striatum and the nucleus accumbens. While the striatum is linked with the inhibition of motion, the nucleus accumbens plays a big role in gratifications (David et al., 2005). The connection between the influence on addictions and the striatum is not as clear as the connection between addictions and the nucleus accumbens. Also alcohol dependence is associated with procedures in the reward centre, the nucleus accumbens as well as in the striatum thus influencing the motor abilities. Since dopamine is looming large in excitation or inhibition in these neuronal areas, the functionality of its receptors altered by different genetic information leads to a difference in effects of alcohol consumption, and by the way the consumption of other drugs too, between individuals being carriers for different alleles. The dopamine receptors can be divided into the D1-, D2-, D3-, D4- and D5-receptor. During the D1- and the D5-receptor activate a stimulating g-protein, the D2-, D3- and D4-receptor lead to the activation of a

inhibiting g-protein (Ishiguro et al., 1998). The locus of receptor expression determines the respective effects of dopamine release into the synaptic gap. The DRD1 is responsible for the dilatation of several blood vessels such as the coronary arteries and brain arteries. The DRD3 and DRD4 are located in the limbic system, thus having influence on the person's emotions which are bear a meaning in behavioural patterns that characterise addictions. The DRD2 is supposed to have the biggest importance for alcohol dependence (Chen et al., 2001). The genetic information for this receptor is located on chromosome 11q22-q23 (Prasad et al., 2010). The receptor is located in the striatum, the nucleus accumbens and the limbic system as well. The genetic polymorphisms of that inhibitory g-protein coupled receptor lead to different efficiencies in information transmission and altered protein synthesis resulting in altered receptor densities in the relative neuronal regions. As several studies stated there exist differentials in receptor densities that correlate with the individual drinking behaviour.

The SNP TaqIA in the ankyrin repeat and kinase domain containing one (ANKK1) gene (DRD2 TaqIA) is the most frequently studied (Lee et al., 2013). The ANKK1 gene is closely linked with the DRD2 gene cluster on chromosome 11q23. The mutations of the ANKK1, of which the TaqIA (Glu713Lys) polymorphism is considered with alcoholism, were believed to be part of the promoter region of the DRD2 gene before but then it was identified to be near the DRD2 and being able to have influence on the expression of that dopamine receptor. Nowadays the polymorphisms of the ANKK1 are thought to play a role in the controlling of dopamine synthesis in the brain and interact with the receptor transcription and translation. So when talking about alcohol dependence and SNPs in DRD2, the TaqIA polymorphism, that you can find 10 kb downstream of the DRD2 gene containing genetic information for a kinase gene named ankyrin repeat and kinase domain contraining 1 (ANKK1), is of outstanding importance for the development of alcohol addiction (Suraj Singh et al., 2013). The serine or threonine kinase that derives from ANKK1 is mostly expressed in placenta and spinal cord RNA (Neville et al., 2004). The actual meaning of the TaqIA polymorphisms on dopamine reception is based on the difference in receptor amounts according to the allele of the TaqIA (TaqIA1 or TaqIA2; Gorwood et al., 2000).

245 alcohol-dependent patients were subtyped by Lesch typology (Lesch et al., 1990) and compared to 110 healthy controls for dopamine receptor D2 polymorphisms linked with alcohol dependence (Lee et al., 2013). They discovered a significant difference in alleles of

the TaqIA between cases and controls. The likeliness of becoming an alcoholic within the group of homozygous carriers for the TaqIA1 allele was higher as without these alleles although the results were not significant for the homozygous TaqIA1 carriers. To unmask alcohol dependent subjects, the examiners processed a semi-structured questionnaire including questions for ethnicity, family histories, age of first alcohol use, duration of alcohol dependence and some more detailed questions about the participants drinking behaviour in the past and the present. Afterwards the AUDIT was performed to achieve comparable and standardized results. After they had taken blood samples of every individual, they genotyped them for DRD2 -141C (rs1799732), a gene in the promoter region of the dopamine receptor D2, exon 8 (rs6276) and ANKK1 TaqIA (rs1800497) by using the polymerase chain reaction with restriction fragment length polymorphisms.

The role of dopamine in psychiatric disorders including alcohol dependence is conveyed by the mesolimbic dopaminergic system that induces self-awarding and thus continuous repeating of the consumed substances that can lead to addictions (Cloninger CR, 1987). This dopaminergic system can be activated by ethanol. The dimension of that process is basically dependent on the amount and functioning of dopamine receptors in the nucleus accumbens and other regions being involved in the neuronal trial of addiction development. Because dopamine is omnipresent in those areas, it is supposed to interact with alcohol consumption that leads to altered transmitter concentrations in the brain.

Of the five dopamine receptors the DRD2 can control the synthesis of dopamine and its release in the synaptic gap (Zigmond et al., 2002). In so doing the DRD2 has a special influence on the central reward pathway and therefore has been linked with the development of alcohol dependence. The above mentioned TaqIA1 allele is associated with a decreased DRD2 density in the nucleus accumbens, the striatum and the mesolimbic system (Cohen et al., 2007). Thus people with the TaqIA1 allele (cytosine) require larger transmitter concentrations to achieve the same rewarding in comparison with those being carrier for the TaqIA2 allele (thymine; Neville et al., 2004). The enlarged transmitter concentrations required to achieve positive award, demand higher amounts of substances inducing positive feedback in the nucleus accumbens and mesolimbic pathway. Ethanol enumerates to those substances inducing positive feelings after consumption.

The DRD2 gene on chromosome 11q22-q23 has been associated with salience attributions and craving in alcoholics and by this means with higher amounts of ethanol consumed (Prasad et al., 2010). To investigate the importance of three SNPs in the dopamine receptor D2 for alcoholism, the scientists analysed 90 north Indian alcoholic subjects and 60 healthy controls that were sex- and age-matched. The three SNPs were -141C Ins/Del in the promoter region of the DRD2 gene (rs1799732), TaqIB 1kb upstream from exon 2 of the DRD2 gene (rs17294542) and TaqIA 10kb downstream from exon 8 of the DRD2 gene (rs1800497). The TaqIA1 allele has been linked with low DRD2 availability in the striatum and because ethanol leads to a higher dopamine release in the ventral striatum inducing positive emotions, alcohol dependent subjects with the TaqIA1 allele need more ethanol for the same outcome. In this manner the dopamine D2 receptor emphasizes its importance as a regulator in brain guerdon mechanisms. The participants were genotyped and their data was analysed with chi-square test and HWE for each of the genetic markers to explore whether they are entailed separately. Although the case and especially the control group size was quite small, we included this study in our meta-analysis because the demographic parameters of the control group containing age, sex and other aspects were very similar to the ones of the cases thus representing a reliable matched-pairs study design as the groups were well comparable.

Ishiguro et al. (1998) analysed the -141C Ins/Del and the TaqIA1/2 polymorphisms of the DRD2 gene in a population of 209 Japanese alcoholics and 152 age- and sex-matched Japanese controls. Both groups consisted nearly exclusively of males. Genomic DNA was prepared from peripheral blood cells by the phenol extraction methods and the two relevant regions of the patients were genotyped using the PCR and RFLP. Then the chi-square test was performed to detect differences in allele frequencies between cases and controls. The homozygous carriers of the TaqIA1 allele showed a slightly increased risk for alcohol dependence compared with other genotypes (OR=1,21).

As a result from these first studies we analysed and after getting to know more about the mechanisms of dopamine receptors, it became clear that the DRD2 may influence the process of becoming an alcoholic less than the maintenance of remaining alcohol dependent by requiring larger and larger amounts of ethanol when there is a genetic vulnerability in

form of a low receptor density that make higher alcohol levels necessary to achieve sufficient reward effects (Cohen et al., 2007).

Referring to another ethnic group, Luo et al. (2005) examined 251 nonalcoholic, unrelated, healthy controls and 200 Mexican American alcoholics for varieties in the DRD2 gene. In this ethnic group the data showed that DRD2 genes, exon 8 A/G and TaqIA1/2 (guanine/adenine) aren't associated with alcohol dependence in a significant way, while -141C Ins/Ins is associated with the disease in this population (OR=1,71; 95%CI: 1,08-2,73). The studied SNPs were all located on chromosome 11q22,3-23,1 and belonged to the DRD2 gene which is made of eight exons and which expands 270 kb of which 250 kb at the beginning form a large intron. The cases fulfilled DSM criteria IV without any other current substance abuse apart from tobacco or coffeine and they did not suffer from a diagnosed mental illness at the moment of examining them. Of the analysed subjects 198 were carrier for the TaqIA1 allele and 252 were carrier of the TaqIA2 allele. 90 persons with the TaqIA1 SNP were alcohol dependent while 110 with the TaqIA2 allele suffered from this addiction too. These numbers show that there does not exist a connection between alcohol dependence and the SNP in that community.

While several studies came to variable results about the impact of the TaqIA1 allele on the likelihood of becoming alcohol dependent (Noble EP, 2003), the study by Berggren et al. (2006) reported a slight over-representation of the DRD2 TaqIA1/1 genotype in the alcohol dependent subjects compared to the control group. The results weren’t statistically significant though (95% CI:0,82-3,19). The TaqIA2/2 genotype however represented a protective factor from alcoholism (OR=0,72, CI=0,55-0,94). They took blood samples from 375 alcohol dependent subjects and from 842 controls and used a timeline of 10 years of alcohol dependence as a basis for the difference between cases and controls.

The allele frequencies of TaqIA polymorphisms of the ANKK1 gene near the dopamine receptor D2 gene were analysed in a community of 115 alcohol-dependent Brazilian males and 114 ethnically matched controls. The examiners did not find out that there exists a clear association of DRD2 TaqIA1 with alcohol dependence. They could even report a protective effect of the TaqIA1/1 genotype towards alcohol dependence without gaining statistically significant data. The problem consisted in the fact that they had only 5 homozygous TaqIA1 carriers in the case sample and 6 in the control sample which made this genotype very rare

and hard to analyse ratios. What is more they could reveal a link between the TaqIA1 allele and psychiatric disorders which are sometimes linked with alcohol addiction (Bau et al., 2000).

209 Japanese alcoholics and 152 age- and sex-matched Japanese controls took part in the analysis by Ishiguro et al. (1998). They had a look after the -141C Ins/Del and the TaqIA1/2 alleles of the DRD2 gene and their importance for the deveopment of alcohol dependence. The TaqIA1 tended to be in a higher frequency among cases than among controls.

Another study with East Asian case and control subjects examined the role of -141C Ins/Del and TaqIA1/2 polymorphisms in the DRD2 gene and alcohol dependence among Meites of Manipur, a Mendelian population of India. They included 129 cases conforming DSM IV criteria for AD and 286 controls in their analysis (Suraj Singh et al., 2013). Cases and controls did not differ significantly in age. Although they kept alluding that education and environmental circumstances have a great influence on the drinking behaviour of each participant and hence may alter the risk of alcoholism inherited by the parental alleles, the TaqIA1/1 seemed to be associated with alcohol dependence (OR=1,62) as dopamine is thought to be the most important neuronal substance for alcohol-related reinforcement as well as gratification behaviour at all (Cohen et al., 2007). The TaqIA site fo the ankyrin repeat and kinase domain containing one (ANKK1) is closely linked to the DRD2 gene on chromosome band 11q23.1 and therefore plays a role in receptor concentration (Neville et al., 2004). During the TaqIA1 allele causes a reduced DRD2 expression in the striatum, the nucleus accumbens and the mesolimbic pathway, the TaqIA2 allele leads to the opposite effect (Suraj Singh et al., 2013). They gained significant results for a negative association between the TaqIA2/2 genotype and alcohol dependence (OR=0,59; 95%CI: 0,37-0,93). The examiners took blood samples to gain information about the genotypes present in the cases and controls. The statistical analysis consisted of the gene counting method and Hardy-Weinberg Equilibrium to test each SNP using POPGENE 1.31. A chi-square test was conducted to judge genotypic counts with alcohol dependent participants. Although they found association between the genotypes and the disease, they also stated a clear association between aspects such as education, work and consumption of other addictive substances and the drinking conduct of the subjects. So it became obvious that persons with lower education, unemployment and smoking consumed more alcohol at an earlier time of their

lives than others regardless from their genotype. This emphasizes again the meaning of epigenetic factors involved in the development of alcohol dependence and addictions in general and the risk that these factors may cause bias our analyses about genotypic predispositions for alcoholism.

Another examination used a screened case-control study design when trying to answer the question if a connection between the TaqIA polymorphisms and alcohol dependence existed. Therefore 160 European American alcohol-dependent subjects and 136 matched EA controls were analysed for their genotype and their drinking behaviour by completing a questionnaire (Gelernter and Kranzler, 1999). They could not report a statistically significant association between the TaqIA1/1 genotype and alcoholism (OR=2,02; 95% CI=0,45-12,36). To expand the possibility of finding a genetic predisposition of the DRD2 gene for alcohol dependence, they also analysed other genetic sequences in the DRD2 gene. But they didn't detect any SNP being statistically significant connected with alcoholism or being linked with a certain behavioural pattern that might bring forward the chance of regular consumption of high amounts of ethanol.

To give an overview about the studies we included that dealt with the TaqIA1/1 genotype (cytosine/cytosine; 713Glu/713Glu) and the risk of becoming alcohol dependent, we summed up the data given in the following table:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Geijer	1994	Sweden	3	5	0,18	0,21	0,34	0,64 (0,09-3,45)
Ishi-guro	1998	Japan	34	24	0,43	0,35	0,37	1,21 (0,65-2,3)
Lee	1999	Taiwan	28	11	0,48	0,39	0,40	1,88 (0,84-4,46)
Gelern-ter	1999	USA	7	3	0,17	0,18	0,46	2,02 (0,45-12,4)
Sander	1999	Germany	11	6	0,18	0,17	0,82	1,16

								(0,38-3,9)
Bau	2000	Brazil	5	6	0,27	0,21	0,59	0,82 (0,19-3,32)
Shaikh	2001	India	8	7	0,42	0,42	0,15	1,25 (0,36-4,43)
Lu	2001	Taiwan	23	11	0,49	0,39	0,41	2,08 (0,89-5,09)
Pasto-relli	2001	Italy	2	2	0,16	0,13	0,34	1,07 (0,07-15,2)
Limosin	2002	France	7	4	0,25	0,20	0,85	1,59 (0,39-7,63)
Foley	2004	Australia	8	4	0,3	0,22	0,47	2,64 (0,68-12,4)
Luo	2005	USA	43	49	0,45	0,44	0,75	1,13 (0,69-1,83)
Berggren	2006	Sweden	17	25	0,22	0,18	0,81	1,63 (0,82-3,19)
Huang	2007	Taiwan	38	67	0,37	0,37	<0,001	1,08 (0,64-1,82)
Wang	2007	Taiwan	18	25	0,47	0,41	0,40	1,74 (0,82-3,62)
Samocho-wiec	2008	Poland	2	4	0,18	0,18	0,63	0,61 (0,05-4,33)
Krasch-ewski	2009	Germany	18	16	0,19	0,18	0,09	1,16 (0,55-2,47)
Prasad	2010	India	4	4	0,27	0,22	0,37	0,65 (0,11-3,65)
Bhaskar	2010	India	20	29	0,46	0,47	0,23	0,97 (0,47-1,97)
Kovanen	2010	Finland	32	15	0,23	0,19	0,29	2,20 (1,14-4,44)

Lu	2010	Taiwan	28	36	0,43	0,36	0,23	1,54 (0,85-2,75)
Schelle-kens	2012	Nether-lands	2	1	0,18	0,15	0,32	1,81 (0,09-108)
Suraj Singh	2013	India	16	23	0,39	0,30	0,47	1,62 (0,77-3,3)
Lee	2013	Korea	49	21	0,48	0,39	0,09	1,48 (0,81-2,78)

[a]Number of cases being carrier for the TaqIA1/1 genotype

[b]Number of controls being carrier for the TaqIA1/1 genotype

[c]Minor allele frequencies for the populations' TaqIA1/1 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the TaqIA1/1 genotype and alcohol dependence

Table 4.1
Characteristics of the studies dealing with carriers for the homozygous TaqIA1/1 genotype given by author, year, country, number of TaqIA1/1 cases and controls, minor allele frequencies, HWE and odds ratios with 95% confidence intervals.

As **Table 4.1** suggests the TaqIA1 (cytosine; 713Glu) allele can be considered the SNP because it is the allele with the minor frequency in the majority of the studies.

We had to exclude some studies because they delivered the information about the association of the TaqIA1/1 genotype of the ANKK1 near the dopamine receptor D2 gene in an idiosyncratic way: Eriksson et al. (2000); Connor et al. (2002); Curtis et al. (1999), Hallikainen et al. (2003), Rowe et al. (1999). On the other hand there were studies without a control group which we could not include in our meta-analysis: Bierut et al. (2000); Wiesbeck et al. (2006); Grzywacz et al. (2012); van der Zwaluw et al. (2011); Dick et al. (2007); Munafo et al. (2005). Another study examined the A1 allele in controls only (Turner et al., 1997). The study by Joe et al. (2008) didn't give any detailed information about the control group, during the control group in the study by Landgren et al. (2011) only counted 32 participants.

Consequently these two studies had to be excluded as well as they didn't meet our inclusion criteria.

Then we collected the data given from studies dealing with the TaqIA1/1 genotype and the risk of becoming alcohol dependent in comparison to the heterozygous genotype and the TaqIA2/2 genotype and created a meta-analysis:

Summary meta-analysis TaqIA1/1 (1)

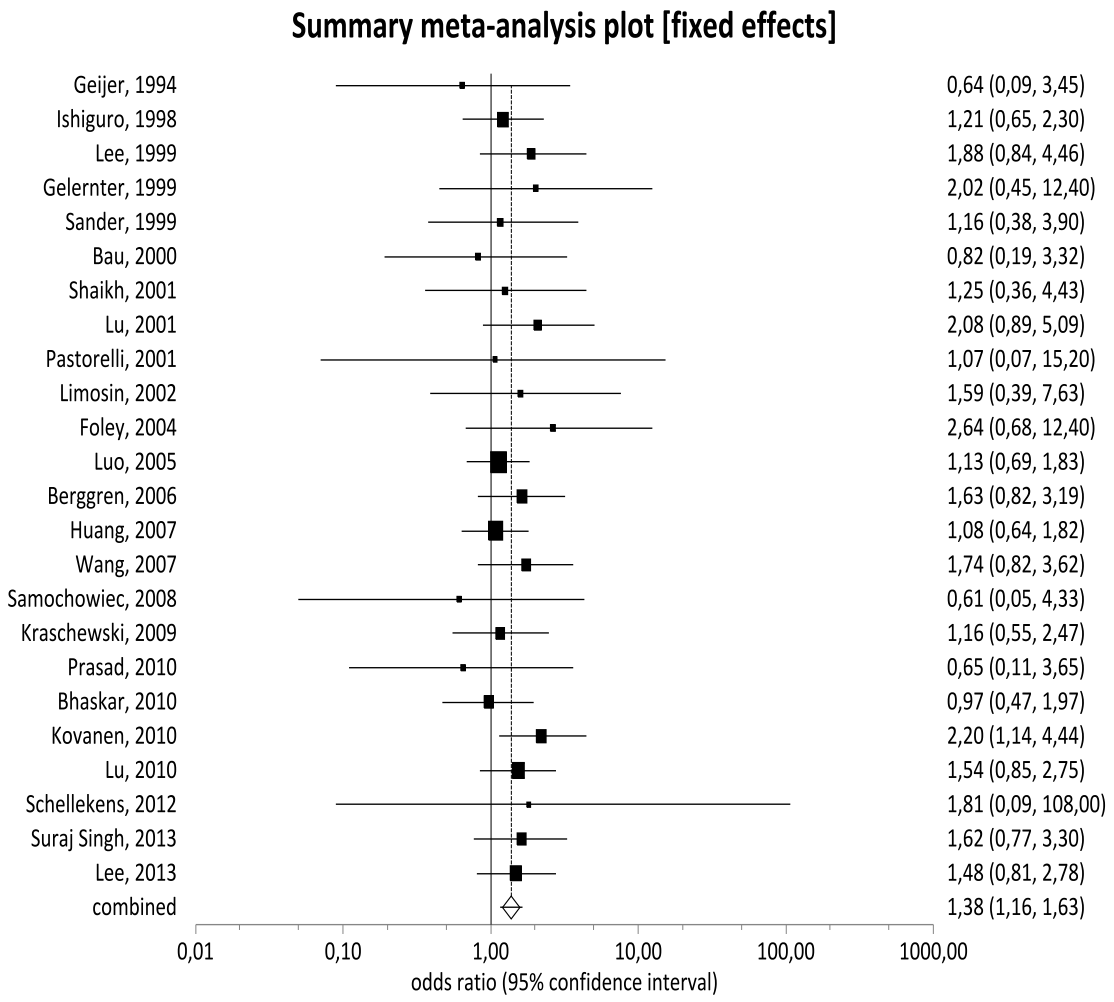


Figure 4.1

Association of the genotype TaqIA1/1 of the ANKK1 near the DRD2 gene with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the TaqIA1/1 (1) meta-analysis: The meta-analysis implied a significantly higher risk for alcohol dependence by being carrier of the TaqIA1/1 gene variant (OR=1,38; 95% CI=1,16-1,63) throughout any ethnicity examined. The individual odds ratios ranged from 0,61 to 2,64. The Cochran Q test (P=0,99) confirmed quite low between-study heterogeneity as well as the Inconsistency-test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel-Method).

The overall effect of this genotype implies a higher risk for becoming alcohol dependent compared to the other genotypes of the TaqIA located on chromosome 11. Several studies reported that effect in Asian populations (Lee et al., 2013; Suraj Singh et al., 2013; Wang et al., 2007; Shaikh et al., 2001; Ishiguro et al., 1998). Also in Caucasian samples there was an association found (Gelernter et al., 1999; Berggren et al., 2006; Limosin et al., 2002). But most of the studies' results did not report a significant association between the TaqIA1/1 genotype and alcoholism. The standard deviation was too big and the number of participants too low to produce small 95% confidence intervals that delivered significant results. Our meta-analysis however put the results by all the studies together and found a significant linkage between the carriers of the TaqIA1/1 genotype and alcoholism. The studies that reported the opposite effect of the TaqIA1/1 genotype for the development of alcohol dependence (Geijer et al., 1994; Bau et al., 2000; Samochowiec et al., 2008) had only less than 10 participants with this genotype in cases and controls. So this genotype was very rare in these populations and in other populations as well, thus representing the single nucleotide polymorphism.

Discussion of the TaqIA1/1 (1) meta-analysis: The TaqIA1 allele is associated with a decreased DRD2 density in the nucleus accumbens, the striatum and the mesolimbic system (Cohen et al., 2007). Therefore people with the homozygous genotype of the TaqIA1 allele require higher amounts of alcohol to achieve rewarding emotions through the dopamine concentration that is increased by ethanol consumption. They have to consume more alcohol than other genotypes of the TaqIA to reach the same stimulation of the nucleus accumbens. When TaqIA1/1 genotypes develop an alcohol addiction, the amount of ethanol they have to consume still rises and is always on top of the required amount of other TaqIA genotypes that are less likely to develop alcohol dependence as the amount of ethanol required to achieve rewarding is lower. So it seems reasonable that the homozygous carriers of the TaqIA1 allele are considered to have a nearly 1,4 fold risk for developing alcohol dependence

in comparison to other TaqIA genotypes. As there are just a few individuals with this genotype, the results are more vulnerable towards disturbing factors as one carrier more or less in either controls or cases can already switch the direction of association completely.

We looked for publication bias in the funnel plot below:

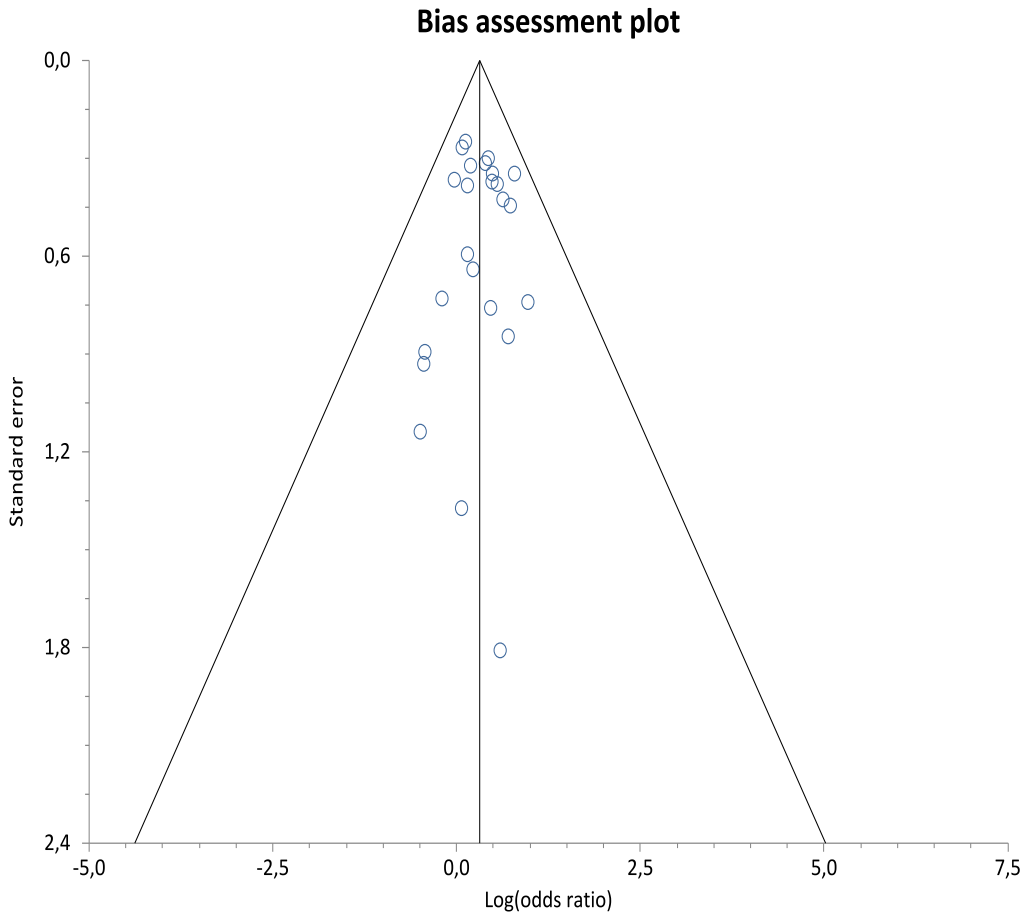


Figure 4.2:
The funnel plot is showing log(OR) and standard error for the association of TaqIA1/1 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,78.

However in **Figure 4.1** we included studies that had quite large 95% CIs thus delivering numbers with a very small force of expression. So we excluded studies with 95% confidence intervals bigger than 8 together with those studies not meeting HWE in the control sample from a second meta-analysis:

Summary meta-analysis TaqIA1/1 (2)

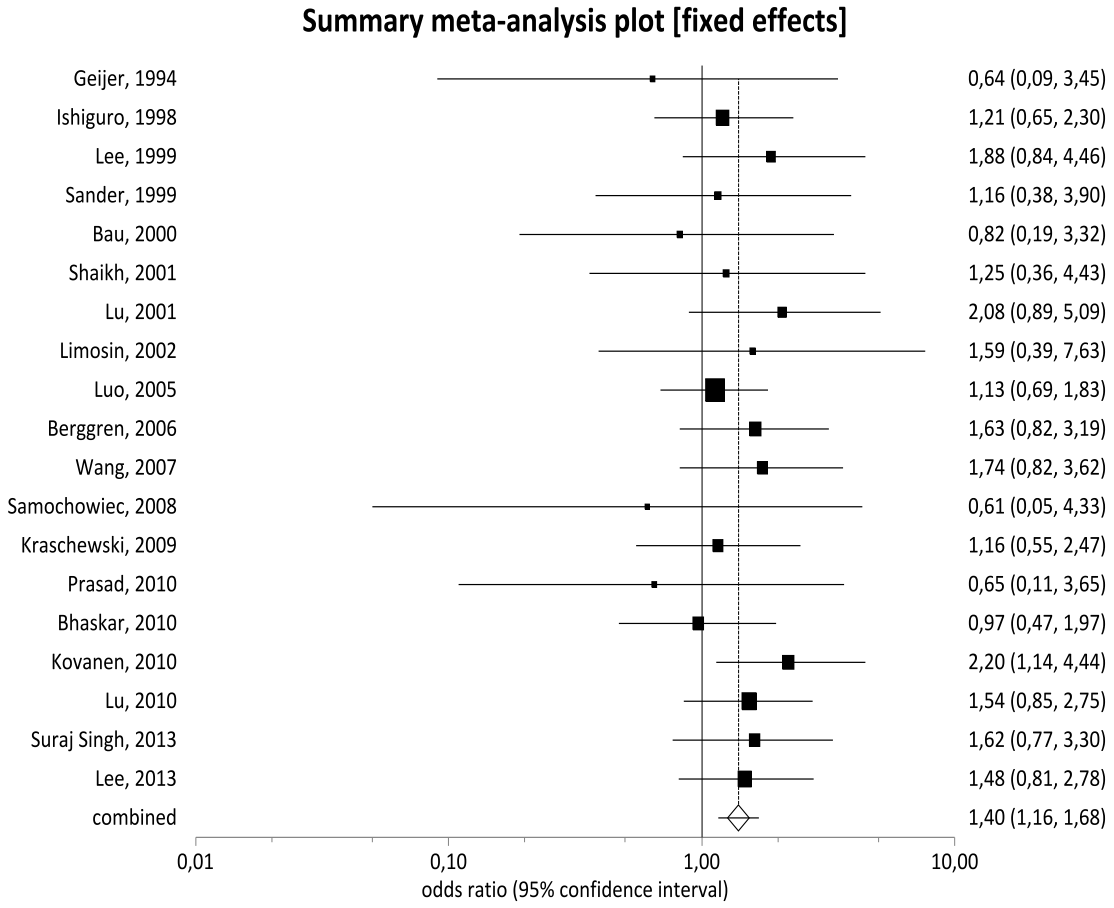


Figure 4.3

Association of the genotype TaqIA1/1 of the ANKK1 near the DRD2 gene with alcohol dependence after excluding some studies for the above mentioned reasons. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the TaqIA1/1 (2) meta-analysis: The meta-analysis implied a significantly higher risk for alcohol dependence by being carrier of the TaqIA1/1 gene variant (OR=1,40; 95% CI=1,16-1,68) throughout any ethnicity examined. The individual odds ratios ranged from 0,61 to 2,20. The Cochran Q test (P=0,98) confirmed quite low between-study heterogeneity as well as the Inconsistency-test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel method).

After excluding several studies because they didn't meet HWE in the controls or they were dealing with a 95% confidence interval larger than 8, hence not delivering useful data, the combined effects still remained the same. So we can consider the TaqIA1/1 genotype to be a high-risk genotype for the development of alcohol dependence in comparison to the heterozygous and the TaqIA2/2 genotypes. From the studies we examined, there was only the study by Kovanen et al. (2010) that showed a statistically significant association between the TaqIA1/1 genotype and alcoholism. Our meta-analysis however put all the studies together that were giving significant numbers deriving from valide and reliable case-control and cohort studies and in so doing we found a significant positive association between the TaqIA1/1 genotype and alcohol dependence throughout Asian and Caucasian populations at least.

As a sensitivity analysis we were looking for publication bias in the following figure:

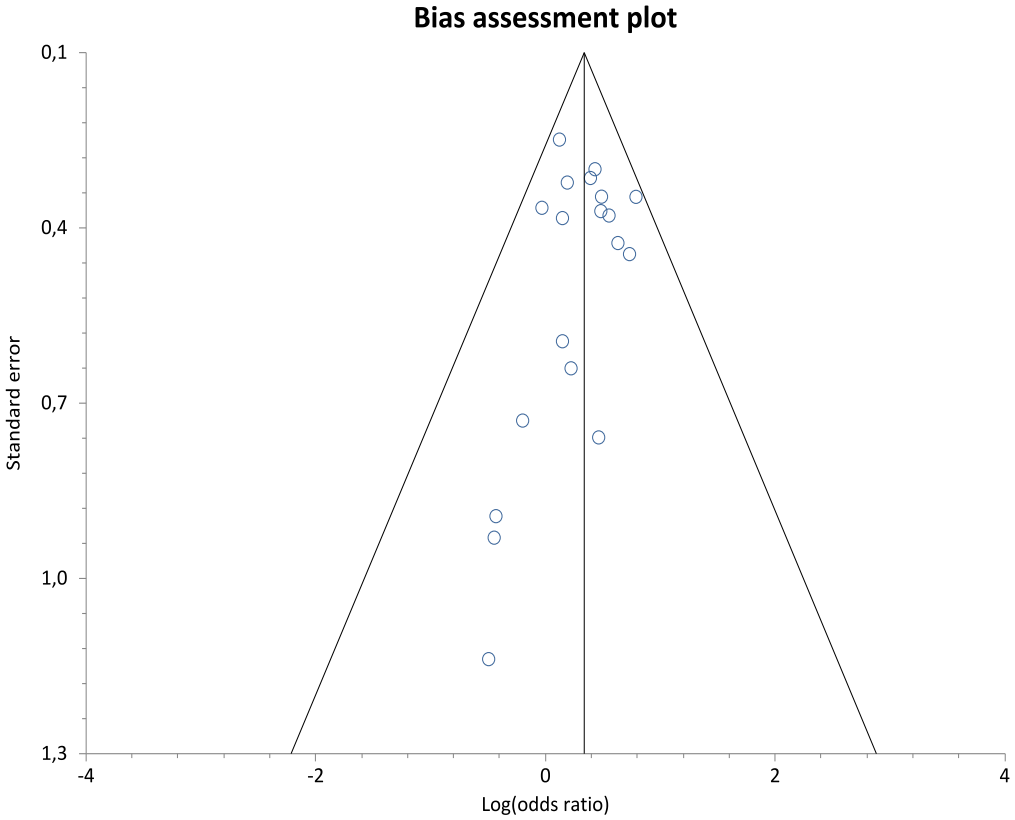


Figure 4.4:
The funnel plot is showing log(OR) and standard error for the association of TaqIA1/1 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,18.

As being homozygous carrier of the TaqIA1 allele from the ANKK1 near the DRD2 gene has influence on the transcription rate of the DRD2 receptor and also on the clinical observed drinking behaviour, we also considered the homozygous TaqIA2 carriers to be associated with alcoholism as the receptor density of the DRD2 is increased when being carrier of this allele (Cohen et al., 2007).

Therefore we collected the data from studies dealing with homozygous carriers of the TaqIA2 allele (thymine/thymine; 713Lys/713Lys):

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Geijer	1994	Sweden	51	52	0,18	0,21	0,34	0,79 (0,39-1,61)
Ishi-guro	1998	Japan	64	67	0,43	0,35	0,37	0,56 (0,35-0,88)
Lee	1999	Taiwan	33	30	0,48	0,39	0,40	0,63 (0,33-1,21)
Gelern-ter	1999	USA	112	91	0,17	0,18	0,46	1,15 (0,68-1,94)
Sander	1999	Germany	211	136	0,18	0,17	0,82	0,94 (0,62-1,41)
Bau	2000	Brazil	58	72	0,27	0,21	0,59	0,59 (0,34-1,04)
Shaikh	2001	India	16	15	0,42	0,42	0,15	1,19 (0,47-3,02)
Lu	2001	Taiwan	22	30	0,49	0,39	0,41	0,54 (0,26-1,08)
Pasto-relli	2001	Italy	43	49	0,16	0,13	0,34	0,77 (0,32-1,87)
Limosin	2002	France	66	68	0,25	0,20	0,85	0,70 (0,39-1,23)
Foley	2004	Australia	43	65	0,3	0,22	0,47	0,86 (0,45-1,61)
Luo	2005	USA	63	81	0,45	0,44	0,75	0,96 (0,63-1,46)
Berggren	2006	Sweden	215	571	0,22	0,18	0,81	0,72 (0,55-0,94)
Huang	2007	Taiwan	65	121	0,37	0,37	<0,001	0,99

								(0,60-1,66)
Wang	2007	Taiwan	23	51	0,47	0,41	0,40	0,96 (0,5-1,82)
Samocho-wiec	2008	Poland	79	100	0,18	0,18	0,63	0,92 (0,54-1,57)
Krasch-ewski	2009	Germany	244	255	0,19	0,18	0,09	0,93 (0,67-1,29)
Prasad	2010	India	46	38	0,27	0,22	0,37	0,61 (0,29-1,24)
Bhaskar	2010	India	26	35	0,46	0,47	0,23	1,08 (0,56-2,08)
Kovanen	2010	Finland	311	330	0,23	0,19	0,29	0,85 (0,65-1,10)
Lu	2010	Taiwan	46	104	0,43	0,36	0,23	0,71 (0,45-1,13)
Schelle-kens	2012	Nether-lands	73	70	0,18	0,15	0,32	0,82 (0,43-1,53)
Suraj Singh	2013	India	45	138	0,39	0,30	0,47	0,59 (0,37-0,93)
Lee	2013	Korea	46	45	0,48	0,39	0,09	0,46 (0,27-0,79)

[a]Number of cases being carrier for the TaqIA2/2 genotype

[b]Number of controls being carrier for the TaqIA2/2 genotype

[c]Minor allele frequencies for the populations' TaqIA2/2 carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the TaqIA2/2 genotype and alcohol dependence

Table 4.2
Characteristics of the studies dealing with carriers for the homozygous TaqIA2/2 genotype given by author, year, country, number of TaqIA2/2 cases and controls, minor allele frequencies, HWE and odds ratios with 95% confidence intervals.

In most of the studies the TaqIA2 allele is more frequent than the TaqIA1 allele. Thus we had more participants being homozygous for the TaqIA2 allele as the data shows. The 95% CIs are smaller than the ones in **Table 4.1** which derives from the large number of participants being homozygous for the TaqIA2 allele. In the following meta-analysis we compared the risk of becoming alcohol dependent when being homozygous carrier of the TaqIA2 allele with the risk of becoming alcohol dependent when being another genotype of the TaqIA of the ANKK1 gene near the DRD2 gene.

We had to exclude some studies because they delivered the information about the association of the TaqIA genotypes of the ANKK1 near the dopamine receptor D2 gene in an idiosyncratic way: Eriksson et al. (2000); Connor et al. (2002); Curtis et al. (1999), Hallikainen et al. (2003), Rowe et al. (1999). On the other hand there were studies without a control group which we could not include in our meta-analysis: Bierut et al. (2000); Wiesbeck et al. (2006); Grzywacz et al. (2012); van der Zwaluw et al. (2011); Dick et al. (2007); Munafo et al. (2005). Another study examined the A1 allele in controls only (Turner et al., 1997). The study by Joe et al. (2008) didn't give any detailed information about the control group, during the control group in the study by Landgren et al. (2011) only counted 32 participants. So these two studies had to excluded as well.

Then we collected the data given by the studies meeting our inclusion criteria and performed our analysis:

Summary meta-analysis TaqIA2/2

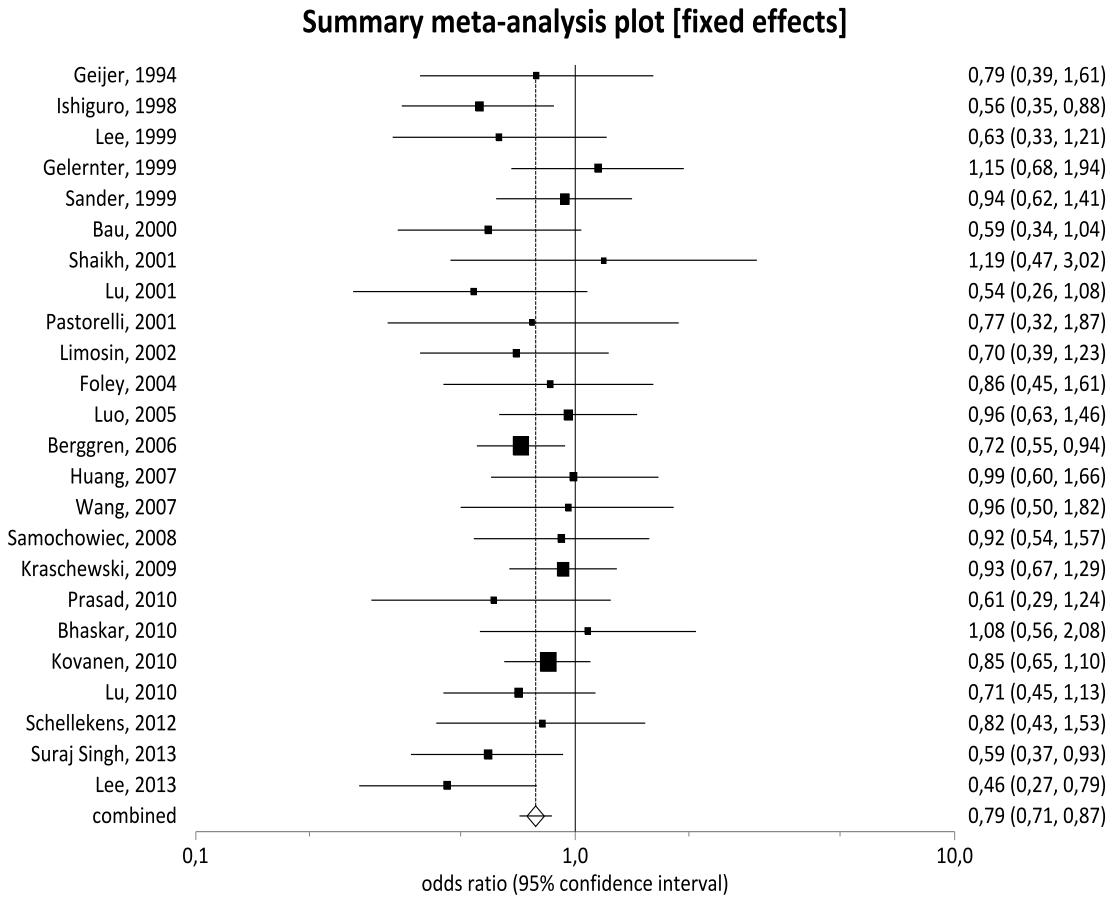


Figure 4.5

Association of the genotype TaqIA2/2 of the ANKK1 near the DRD2 gene with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the TaqIA2/2 meta-analysis: The meta-analysis implied a significantly decreased risk for alcohol dependence by being carrier of the TaqIA2/2 gene variant (OR=0,79; 95% CI=0,71-0,87) throughout any ethnicity examined. The individual odds ratios ranged from 0,46 to 1,19. The Cochran Q test (P=0,68) confirmed quite low between-study heterogeneity as well as the Inconsistency-test (I^2=0%). This led to the application of a fixed effects model (Mantel-Haenszel method).

TaqIA2 homozygosity seems to be a significantly protective genetic constellation for the

development of alcohol dependence as the forest plot clearly reveals a negative association between this genotype and alcohol dependence. There is no study that is dealing with a tiny amount of participants with this genotype as it was the case with the TaqIA1/1 genotype. Thus the 95% confidence intervals are all quite small revealing useful data. The majority of the studies reported a negative association between the TaqIA2/2 genotype and alcohol dependence without giving significant numbers (Kovanen et al., 2010; Prasad et al., 2010; Lee et al., 1999; Lu et al., 2001; Limosin et al., 2002). Only a few studies gave significant results though (Lee et al., 2013; Suraj Singh et al., 2013; Berggren et al., 2006; Ishiguro et al., 1998). After all the combined effect stated a significant association between the TaqIA2/2 genotype of the ANKK1 gene which is associated with an increased amount of dopamine D2 receptors especially in the nucleus accumbens, the striatum and the mesolimbic pathway (Prasad et al., 2010). This association was found for any of the ethnicities examined which were particularly Asians and Caucasians. Anyway this allele seems to be more frequent among Asian and Caucasian populations that were the majority of the subjects the studies we included in our meta-analysis dealt with.

Discussion of the TaqIA2/2 meta-analysis: The association is even stronger than the association between the TaqIA1/1 and a higher risk of becoming alcohol dependent which might be caused by the fact that the TaqIA2 allele appears to be the wildtype allele as it is more frequent in nearly all of the studies' populations, hence being less vulnerable towards disturbing factors. The protection is caused by a higher availability of dopamine D2 receptors in neuronal rewarding systems that lead to a lower amount of alcohol required to benefit from positive feelings (Suraj Singh et al., 2013). So carriers of the TaqIA2/2 genotype don't need to consume as high amounts of ethanol as other genotypes to achieve the same reward feelings. Together with the results of our meta-analysis about the association between TaqIA1/1 carriers with alcoholism this meta-analysis stresses the importance of the dopamine D2 receptor for the central nervous impact of ethanol on the human organism. In contrast to the GABA-A receptors, the polymorphisms we analyzed for the DRD2 receptor didn't alter the structure and thus the opening mechanisms of the receptor. The polymorphisms of the TaqIA lead to a different amount of DRD2 receptors (Suraj Singh et al., 2013). They didn't alter the opening likeliness of the individual receptor. But in the end the results end up in the same effect, either the certain genotype requires a higher amount of transmitters to achieve a certain cellular result or a lower amount. In the case of GABA-A

receptors, the association between the SNPs and the opening mechanisms are not that clear yet (Enoch et al., 2008), in the case of the DRD2 receptor density, the association is clarified. The single nucleotide polymorphism of the TaqIA, the TaqIA1 allele, is causing a lower availability of DRD2 receptors and higher dopamine concentrations needed to achieve the same cellular results compared with other genotypes (Prasad et al., 2010). Hence the likeliness of regular consumption of large amounts of alcohol rises. The TaqIA2/2 protects from alcohol dependence.

We were looking for publication bias in the studies about the association between the TaqIA2/2 genotype and alcoholism:

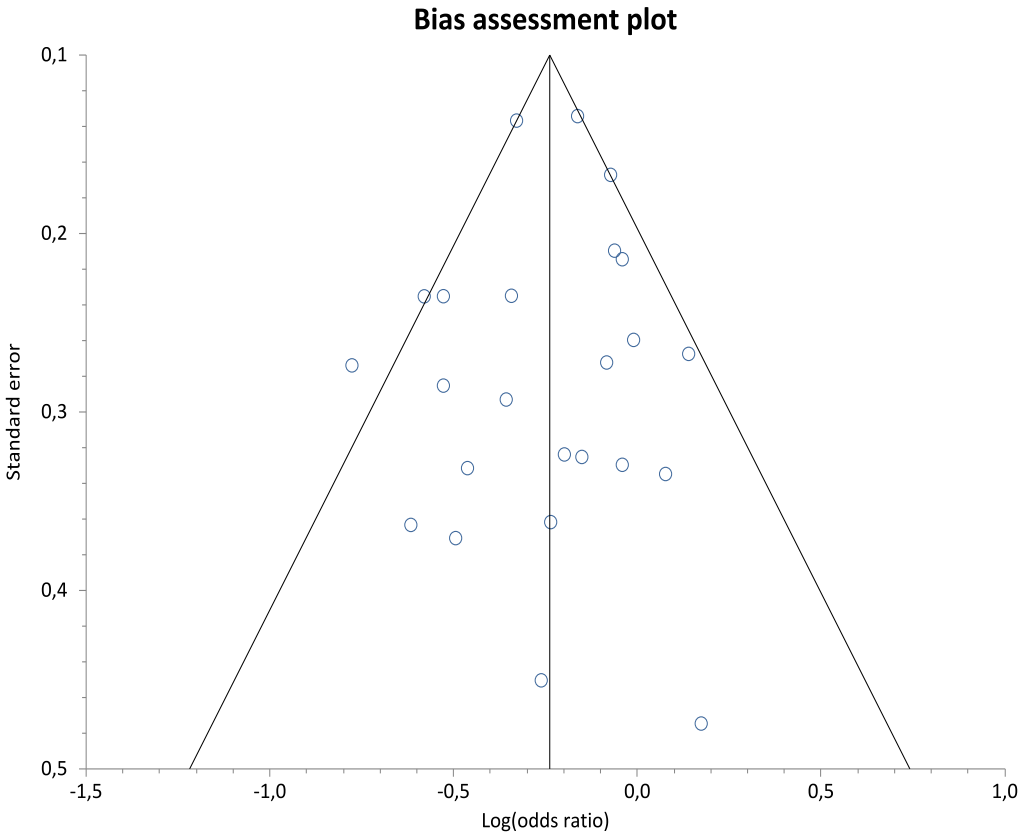


Figure 4.6:
The funnel plot is showing log(OR) and standard error for the association of TaqIA2/2 with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,70.

We had only one study not meeting HWE in the control group (Huang et al., 2007) and all the 95% CIs were quite small, indicating a quite large case and control group size and a small standard deviation. Therefore we didn't have to create a second forest plot leaving out several studies that did not deliver reliable data.

Although the SNP in the TaqIA of the ANKK1 gene has been linked with an influence on alcoholism by altering the density of dopamine receptor D2 in the brain in a variety of studies, it has not been the only polymorphism considered in dopamine reception that has been connected with the complex disease of alcohol addiction. Another not quite as well-studied polymorphism refers to the promoter region of the DRD2 gene. The SNP we focused on produces an insertion or a deletion of a cytosine at position -141 in a part of the promoter region of the DRD2 gene, -141C Ins/Del (rs1799732) causing the amino acid exchange Cys311Ser. This either leads to an increased or a decreased receptor density (Arinami et al., 1997). The Ins allele has been found to lead to a lower receptor amount (Ishiguro et al., 1998).

To support the findings of the case-control studies mentioned below, we also gained information from animal experiments revealing that ethanol may boost the neuronal activity of dopaminergic neurons in the ventral tegmental area (Brodie et al., 1990) and increases the dopamine release in the nucleus accumbens (Weiss et al., 1993). Now it is important how many of the dopamine receptors D2 are available to conduct electric information. The density is altered by the alleles of the TaqIA of the ANKK1 near the DRD2 receptor gene as we already said above but also by the alleles of the -141C in the promoter region of the DRD2 gene.

Lee et al. (2013) performed a semi-structured interview with detailed questions about the participants' ethnic background, their family histories of drinking, their age of the first alcohol use and the duration of alcohol dependence to separate drinkers from non-drinkers. After that they operated a genetic analysis with the individuals' peripheral blood, processing a PCR and RFLP for the dopamine receptor D2 -141C (rs1799732), exon 8 (rs6276) and ANKK1 TaqIA (rs1800497). The statistical analysis included chi-square test or student's t-test

as well as the HWE for each genetic marker. Particularly the association of the -141C Ins/Ins of the DRD2 gene with alcohol dependence had been observed (OR=2,73; 95% CI: 1,64-4,59). The low-risk genotype then was considered to be the -141C Del/Del, but didn't show a significant association with alcohol dependence in our statistical analysis (OR=0,76; 95% CI: 0,23-2,76).

Another examination with 90 alcohol dependence cases and 60 age-matched controls was performed to clarify the role of the -141C SNPs regarding the risk of becoming alcohol addicted. Therefore the scientists started an in vitro analysis to show off that -141C Ins/Del polymorphisms of the DRD2 gene lead to differences in the transcriptional activity and hence to altered receptor expression by lowered or increased production (Prasad et al., 2010). A clinical investigation already stated that a low receptor density and function was found among alcoholics, thus leading to symptoms like craving, subsequent relapse and the need of large ethanol amounts to satisfy the addiction (Tupala et al., 2004). So the impact of -141C Ins/Ins on the likeliness of becoming alcohol dependent is thought to be quite similar to the one of the TaqIA1/1 genotype of the ANKK1 gene. The homozygous -141C Del/Del SNP seemed to be linked with a protection from the disease (OR=0,19; CI=0,06-0,58).

Ishiguro et al. (1998) also reported an association between the -141C Ins in the promoter region of the DRD2 gene and alcohol dependence in an Asian population of 209 Japanese cases and 152 ethnically and age-matched controls that were nearly just males. After the subjects had been genotyped and statistically analysed using HWE for deviations from genotype counts and chi-square test for differences in allele frequencies between cases and controls, the -141C Ins/Ins was significantly increased in alcoholics (OR=1,73; CI=1,08-2,78).

Another community of East Asian subjects presented data that did not show a significantly increased or decreased risk of the -141C Del/Del genotype of the DRD2 gene for alcoholism (OR=1,69; 95% CI: 0,47-5,7). The population consisted of 129 cases meeting DSM IV criteria for alcohol dependence and 286 matched controls. The examiners analysed the meaning of the TaqIA SNPs and the -141C polymorphisms for the development of alcoholism (Suraj Singh et al., 2013). They used lymphocyte DNA for their analysis. After counting the genetic frequencies, Pearson's chi-square test was conducted to evaluate these counts. Surprisingly

the -141C Del/Del genotype delivered numbers showing a linkage with alcohol dependence and the -141C Ins/Ins genotype which was supposed to be an important risk factor for alcoholism by the other studies mentioned above didn't. As a conclusion Suraj Singh et al. (2013) asserted that being carrier for the TaqIA1/1 genotype and the -141C Del/Del genotype of the dopamine receptor D2 leads to the highest risk of becoming alcohol dependent particularly when the first alcohol consumption was at a very early age. The results for the -141C Ins/Del polymorphism stand in contrast to other studies that describe the insertion as a risk and the deletion of that part of the promoter region as a protective factor according to the higher amounts of ethanol consumption needed (Ishiguro et al., 1998; Prasad et al., 2010; Lee et al., 2013; Luo et al., 2005).

Luo et al. (2005) analysed 3 SNPs of the DRD2 gene including -141C Ins/Del, TaqIA1/2 and exon 8 on chromosome 11q22,3-q23,1. They took blood samples from 251 nonalcoholic, unrelated, healthy controls and 200 alcohol-dependent Mexican Americans. After genotyping the participants, the individuals underwent a questionnaire. Only DSM criteria IV patients were included in the case group. The results of the -141C polymorphisms counted 159 alcoholics being homozygous carriers of the -141C Ins allele and only 7 alcohol-dependent subjects being carrier for the -141C Del/Del genotype. These results stated an association between the -141C Ins/Ins genotype with alcoholism but also a positive association between the -141C Del/Del genotype with alcoholism (OR=1,48). This however might have to do with the tiny amount of cases and controls being carrier for the -141C Del/Del alleles, which leads to the problem that the results can easily be blurred.

228 cases with withdrawal symptoms and 215 sex- and ethnicity-matched controls among 4 aboriginal groups (Atayal, Ami, Bunun and Paiwan) and Han Chinese in Taiwan had been examined to study the connection between functional polymorphisms in the promoter region of the dopamine receptor D2 gene (-141C Ins/Del) and the amount of tandem repeats at the 3' untranslated region of the dopamine receptor gene being responsible for altered DRD2 density and alcohol dependence (Chen et al., 2001). The cases included in that examination all met DSM III criteria. To be able to differ between unequal ethnic groups, subject from inter-ethnic marriage were not allowed to participate in the analysis. We calculated the risk of the genotypes for becoming alcohol dependent for each of the

subgroups first and then calculated the overall risk of the whole aboriginal population of the study.

The studies' results suggested that there does not exist a clear association between the -141C Ins/Ins genotype and alcohol dependence in these ethnic groups. In some groups (Ami, Bunun) the polymorphism shows even protective effects as it lowers the amounts of intaken ethanol. The problem with this data might be that there are very little group sizes with the carriers of the -141C Del/Del alleles of the promoter region of the DRD2 gene. The small group sizes lead to a low reliability and validity of the results as the size of the 95% confidence intervals suggests. That's why we also investigated the meaning of the -141C genotypes for all the aboriginal groups together. But still we could only deal with one case subject being homozygous for the -141C Del allele. However it is clear that this case-control study differs in results from other studies including animal analyses that state an association between the -141C Ins/Ins of the DRD2 gene, reduced receptor densities and altered drinking behaviour in rats (Myers and Robinson et al., 1999).

We collected the data given by the studies included about the meaning of the -141C Ins/Ins (cytosine/cytosine; 311Cys/311Cys) genotype for the development of alcohol dependence in the following table:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Ishiguro	1998	Japan	153	93	0,14	0,23	0,09	1,73 (1,08-2,78)
Gelern-ter	1999	USA	123	119	0,12	0,06	0,43	0,47 (0,24-0,92)
Sander	1999	Germany	260	163	0,09	0,09	0,06	1,05 (0,63-1,74)
Chen (all 4)	2001	Taiwan	139	136	0,07	0,08	<0,001	0,81 (0,37-1,75)
Chen (Atayal)	2001	Taiwan	31	27	0,11	0,22	<0,001	1,28 (0,38-4,44)

Chen (Ami)	2001	Taiwan	21	20	0,1	0,07	0,74	0,63 (0,09-3,77)
Chen (Bunun)	2001	Taiwan	53	54	0,04	0,04	<0,001	0,74 (0,1-4,59)
Chen (Paiwan)	2001	Taiwan	34	35	0,03	0	0,86	-
Chen (Chinese)	2001	Taiwan	57	50	0,1	0,11	0,32	1,22 (0,48-3,13)
Luo	2005	USA	159	174	0,12	0,17	0,69	1,71 (1,08-2,73)
Samo-chowiec	2008	Poland	98	124	0,11	0,09	0,5	0,85 (0,44-1,66)
Du	2009	USA	270	235	0,15	0,16	0,26	1,24 (0,88-1,75)
Krasch-ewski	2009	Germany	298	290	0,09	0,11	0,21	1,29 (0,87-1,91)
Prasad	2010	India	55	34	0,23	0,35	<0,001	1,2 (0,58-2,45)
Suraj Singh	2013	India	75	193	0,23	0,18	0,71	0,67 (0,42-1,05)
Lee	2013	Korea	130	49	0,18	0,31	0,06	2,73 (1,64-4,59)

[a]Number of cases being carrier for the -141C Ins/Ins genotype

[b]Number of controls being carrier for the -141C Ins/Ins genotype

[c]Minor allele frequencies for the populations' -141C Ins/Ins carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the -141C Ins/Ins genotype and alcohol dependence

Table 4.3
Characteristics of the studies dealing with carriers for the homozygous -141C Ins/Ins genotype given by author, year, country, number of -141C Ins/Ins cases and controls, minor allele frequencies, HWE and odds ratios with 95% confidence intervals (all 4 means all the aboriginal samples put together into one case and one control group).

We had to exclude some studies either because the authors delivered the data in an idiosyncratic way (Eriksson et al.,2000; Hallikainen et al., 2003; Spitz et al.,1998; Connor et al., 2007) or the studies didn't deal with a control group (Bierut et al., 2000; Blomqvist et al., 2000; van der Zwaluw et al. (2011); Dick et al. (2007)).

Then we created a meta-analysis to compare our results of the statistically comparable papers dealing with the meaning of the -141C Ins/Ins genotype of the dopamine receptor D2 gene compared with the other genotypes of this part of the promoter region which is considered with an alteration in DRD2 density (Arinami et al., 1997):

Summary meta-analysis -141C Ins/Ins (1)

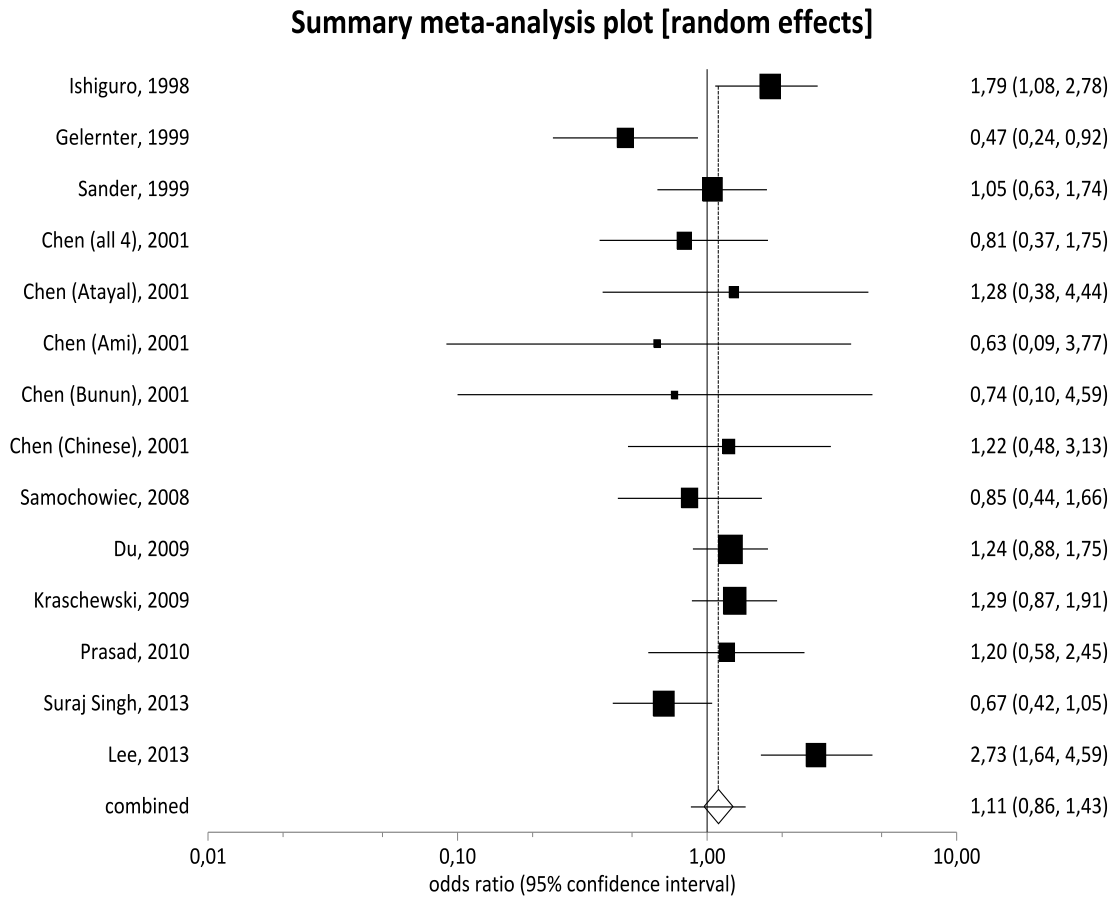


Figure 4.7

Association of the -141C Ins/Ins genotype of the promoter region of the DRD2 gene with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the -141C Ins/Ins (1) meta-analysis: The meta-analysis implied no significantly altered risk for alcohol dependence by being carrier of the -141C Ins/Ins gene variant (OR=1,11; 95% CI=0,86-1,43) throughout any ethnicity examined. The individual odds ratios ranged from 0,47 to 2,73. The Cochran Q test (P=0,006) confirmed quite high between-study heterogeneity as well as the Inconsistency-test (I^2=55,5%). This led to the application of a random effects model (DerSimonian-Laird).

There doesn't exist a higher risk for becoming alcohol dependent when being carrier of the homozygous -141C Ins allele. The only three studies that gave significant results between the genotype and alcoholism were Ishiguro et al. (1998), Gelernter et al. (1999) and Lee et al. (2013). All the other studies did not find a significant association between the -141C Ins/Ins and alcohol dependence. Even those delivering significant results, do report opposite directions of association. During Ishiguro et al. (1998) and Lee et al. (2013) report a bringing forward effect of this genotype towards the development of alcoholism, the study by Gelernter et al. (1999) stated a protective effect.

Discussion of the -141C Ins/Ins (1) meta-analysis: As the -141C Ins/Ins genotype is considered to decrease the amount of DRD2 receptors in the nucleus accumbens and the striatum (Ishiguro et al., 1998), the actual meaning of this polymorphism on the individual drinking behaviour is not clinically significant (OR=1,11; 95% CI=0,86-1,43). What is more the allele frequencies suggest that the -141C Ins allele represents the wildtype allele as one can find it in a much higher amount than the -141C Del allele in all the populations. The also high amount of carriers of the homozygous -141C Ins allele leaves us with the fact that the data we calculated for the association between the -141C Ins/Ins and the development of alcoholism is quite reliable, as we dealt with 95% confidence intervals that gave useful numbers not being too large. This stands for sufficiently big group sizes and quite small standard errors. Nevertheless we had to create a second forest plot, leaving out the studies that didn't meet HWE in the control group as well as the data about the aboriginal subgroups in the study by Chen et al. (2001) which were only composed of a small number of participants.

First we were looking for publication bias as a sensitivity analysis:

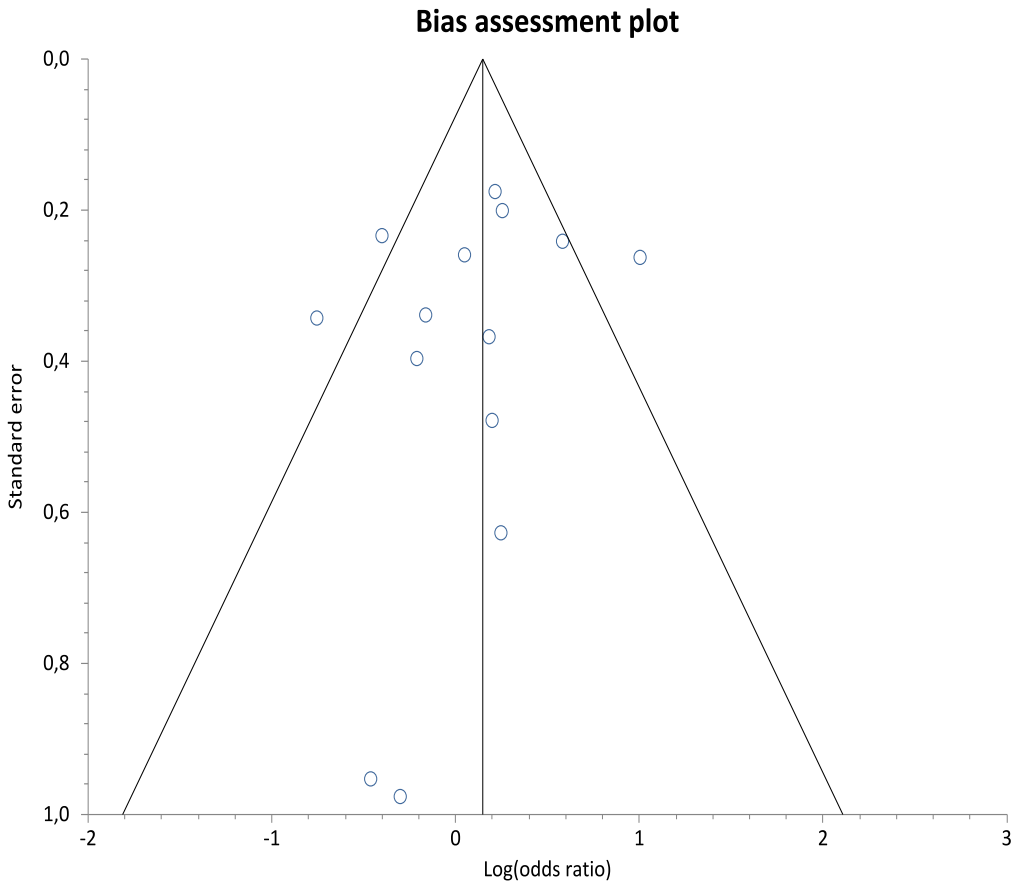


Figure 4.8
The funnel plot is showing log(OR) and standard error for the association of -141C Ins/Ins with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,40.

Summary meta-analysis -141C Ins/Ins (2)

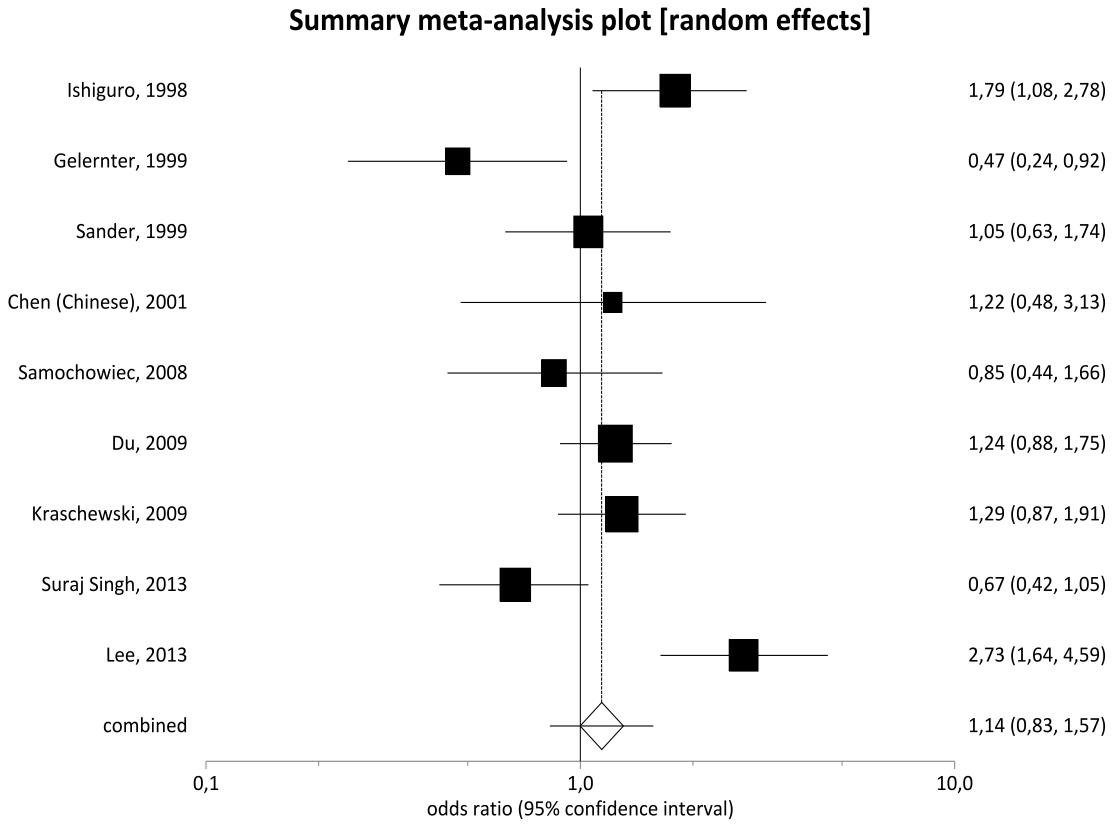


Figure 4.9

Association of the genotype -141C Ins/Ins of the promoter region of the DRD2 gene with alcohol dependence after excluding several studies because they didn't meet HWE in the controls or the number of participants for each aboriginal subgroup in the study by Chen et al. (2001) was too small. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the -141 Ins/Ins (2) meta-analysis: The meta-analysis implied no significantly altered risk for alcohol dependence by being carrier of the -141C Ins/Ins gene variant (OR=1,14; 95% CI=0,83-1,57) throughout any ethnicity examined. The individual odds ratios ranged from 0,47 to 2,73. The Cochran Q test ($P=5x10^{-4}$) confirmed quite high between-study heterogeneity as well as the Inconsistency-test ($I^2=71,1\%$). This led to the application of a random effects model (DerSimonian-Laird).

There were several ethnicities examined for an association of the -141C Ins/Ins genotype with alcoholism, such as Asian (Suraj Singh et al., 2013; Lee et al., 2013; Ishiguro et al., 1998; Chen et al., 2001), Caucasian (Samochowiec et al., 2008; Kraschewski et al., 2009; Sander et

al., 1999) and Mexican American (Du et al., 2009). Throughout any of these ethnicities there was most frequently no association between the homozygous -141C Ins allele carriers and alcohol dependence found. Only the studies by Lee et al. (2013) and Ishiguro et al. (1998) found a positive association between the homozygosity for the wildtype allele of the -141C of the promoter region of the DRD2 gene and alcoholism. Suraj Singh et al. (2013) and Chen et al. (2001) didn't find this association examining participants of the same ethnic background. After all the combined effect revealed that there doesn't exist a clear connection between the -141C Ins/Ins genotype and alcohol dependence in the ethnic groups examined, also after leaving out the studies we excluded from the second meta-analysis.

We were looking for publication bias in the following funnel plot:

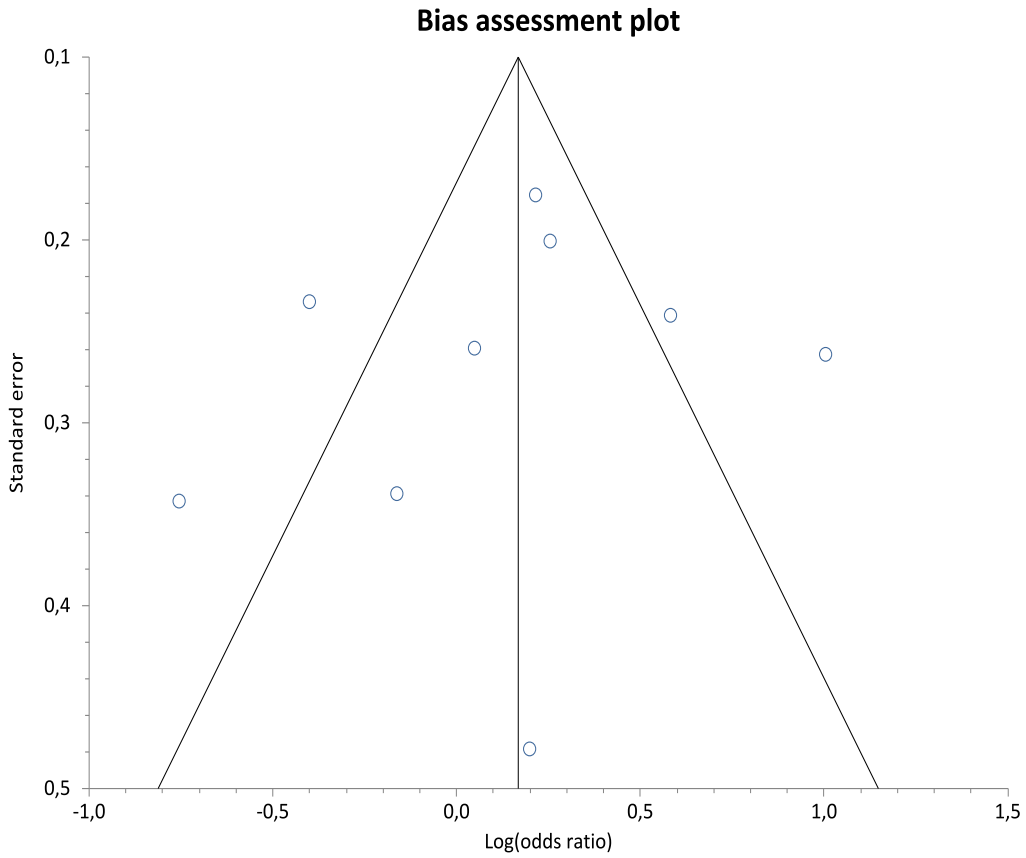


Figure 4.10
The funnel plot is showing log(OR) and standard error for the association of -141C Ins/Ins with alcohol dependence after excluding some studies for the reasons mentioned above. The

bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,56.

As the -141C Del allele of the DRD2 gene leads to deletion of a cytosine in the promoter region, it has also been considered to alter the transcription rate and thus the amount of dopamine D2 receptors produced (Arinami et al., 1997). Ishiguro et al. (1998) reported that the -141C Del allele should increase the amount of DRD2 produced. In our meta-analysis we calculated odds ratios and 95% confidence intervals for the risk of becoming alcohol dependent when being -141C Del/Del genotype in comparison with the other genotypes of this promoter region of the DRD2 gene. Thus we hoped to get insight in the issue if the -141C gene plays an important role in the development of alcohol dependence.

First we collected the data about the -141C Del/Del (-/-; 311Ser/311Ser) genotype given by the studies we included because they met our inclusion criteria and summed the numbers up in the following table:

Author	Year	Country	n[a] (cases)	n[b] (controls)	MAF[c] (cases)	MAF[c] (controls)	HWE[d] (p-value)	OR (95% CI)[e]
Ishiguro	1998	Japan	4	12	0,14	0,23	0,09	0,23 (0,05-0,77)
Gelern-ter	1999	USA	1	0	0,12	0,06	0,43	-
Sander	1999	Germany	5	4	0,09	0,09	0,06	0,78 (0,16-4,02)
Chen (all 4)	2001	Taiwan	1	8	0,07	0,08	<0,001	0,11 (0,003-0,87)
Chen (Atayal)	2001	Taiwan	0	7	0,11	0,22	<0,001	-

Chen (Ami)	2001	Taiwan	0	0	0,1	0,07	0,74	-
Chen (Bunun)	2001	Taiwan	1	1	0,04	0,04	<0,001	1,00 (0,01-79,9)
Chen (Paiwan)	2001	Taiwan	0	0	0,03	0	0,86	-
Chen (Chinese)	2001	Taiwan	0	0	0,1	0,11	0,32	-
Luo	2005	USA	7	6	0,12	0,17	0,69	1,48 (0,42-5,42)
Samo-chowiec	2008	Poland	2	2	0,11	0,09	0,5	1,23 (0,08-17,23)
Du	2009	USA	11	6	0,15	0,16	0,26	1,72 (0,57-5,72)
Krasch-ewski	2009	Germany	6	2	0,09	0,11	0,21	3,11 (0,55-31,7)
Prasad	2010	India	6	16	0,23	0,35	<0,001	0,19 (0,06-0,58)
Suraj Singh	2013	India	6	8	0,23	0,18	0,71	1,69 (0,47-5,70)
Lee	2013	Korea	8	6	0,18	0,31	0,06	0,76 (0,23-2,76)

[a]Number of cases being carrier for the -141C Del/Del genotype

[b]Number of controls being carrier for the -141C Del/Del genotype

[c]Minor allele frequencies for the populations' -141C Del/Del carriers

[d]Hardy-Weinberg-Equilibrium (p-value)

[e]Odds Ratio and 95% Confidence Interval for the association of the -141C Del/Del genotype and alcohol dependence

Table 4.4
Characteristics of the studies dealing with carriers for the homozygous -141C Del/Del genotype given by author, year, country, number of -141C Ins/Ins cases and controls, minor allele frequencies, HWE and odds ratios with 95% confidence intervals (all 4 means all the aboriginal samples put together into one case and one control group).

The table already reveals that the -141C Del/Del genotype is quite rare representing the single nucleotide polymorphism. In some studies it is even that rare that there is no calculation of ORs and 95% CIs possible because there is no carrier of this genotype. Furthermore some of the studies for which we could calculate odds ratios and confidence intervals only dealt with a very low number of -141C Del/Del genotypes which made the 95% CI very large and thus the results quite useless. To be able to analyze the meaning of the -141C Del/Del genotype for the development of alcohol dependence in an adequate way, we created the following forest plot:

Summary meta-analysis -141C Del/Del (1)

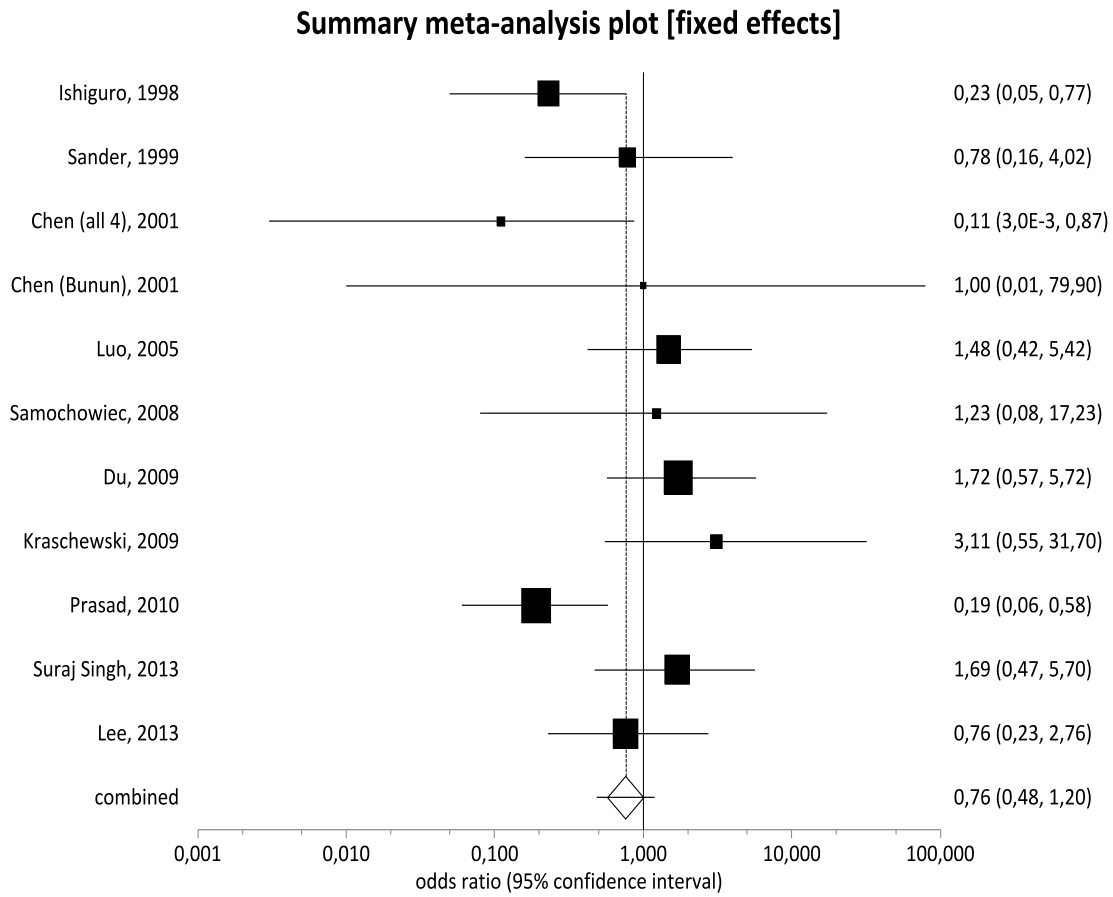


Figure 4.11
Association of the -141C Del/Del genotype of the promoter region of the DRD2 gene with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the -141C Del/Del (1) meta-analysis: The meta-analysis implied no significantly altered risk for alcohol dependence by being carrier of the -141C Del/Del gene variant (OR=0,76; 95% CI=0,48-1,20) throughout any ethnicity examined. The individual odds ratios ranged from 0,11 to 3,11. The Cochran Q test (P=0,07) confirmed quite low between-study heterogeneity as well as the Inconsistency-test (I^2=41,2%). This led to the application of a fixed effects model (Mantel-Haenszel method).

Only Ishiguro et al. (1998) and Prasad et al. (2010) reported a significantly negative association between the -141C Del/Del genotype and alcohol dependence, Prasad et al. (2010) however without meeting HWE in the control sample. All the other studies did not deliver an association between the -141C Del/Del genotype, that leads to a deletion of cytosine in the promoter region of the DRD2 gene, and alcoholism.

Discussion of the -141C Del/Del (1) meta-analysis: Together with the results from the association between the homozygous carriers of the -141C Ins allele, these results suggest that there is no alteration in the risk of becoming alcohol dependent caused by the polymorphisms of this genetic region on chromosome 11. The reported effect of the -141C Del/Del genotype of the promoter region of the DRD2 gene leading to a higher density of dopamine receptors D2 (Ishiguro et al., 1998) doesn't influence the clinical risk of becoming alcohol dependent in a significant way. Hence it doesn't seem to have such a big influence on the receptor density because as we found out in our meta-analysis about the TaqIA polymorphisms, the receptor density of the DRD2 is important for the individual drinking behaviour.

As a sensitivity analysis we were looking for publication bias:

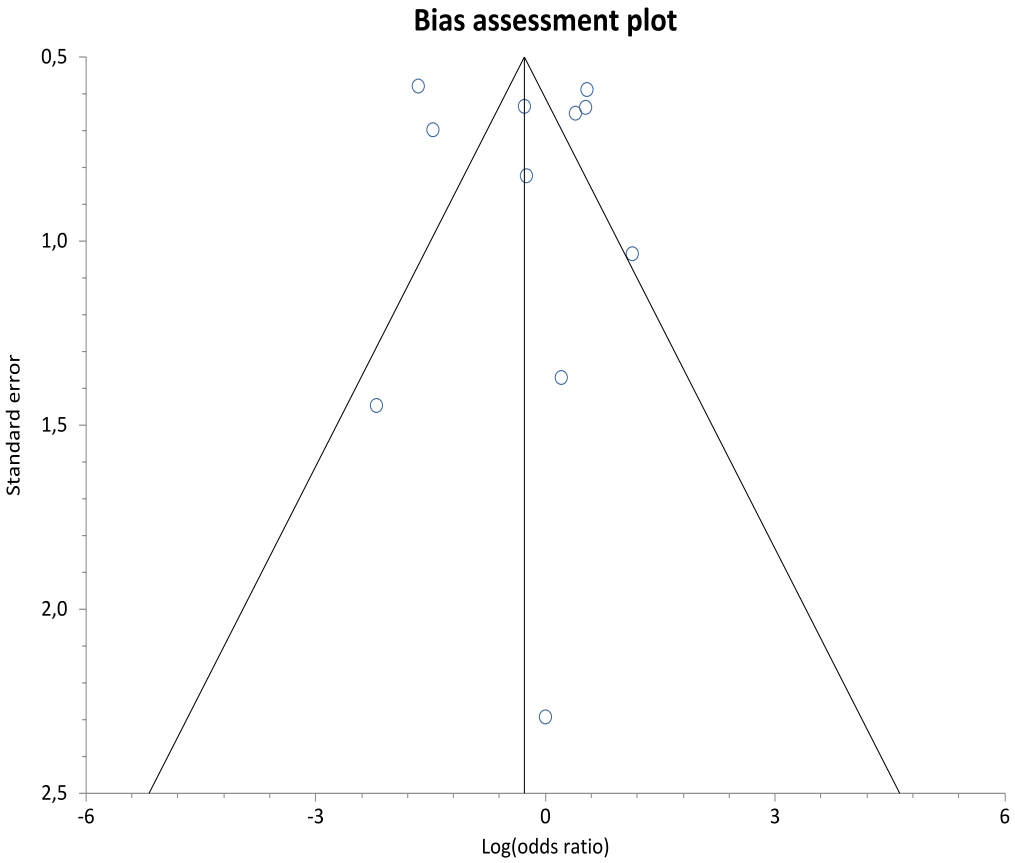


Figure 4.12
The funnel plot is showing log(OR) and standard error for the association of -141C Del/Del with alcohol dependence. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,97.

The fact that the -141C Del allele is quite rare in most of the analyzed populations as one can see in **Table 4.4** leads to quite large 95% CIs for several studies. This again causes the problem of revealing quite useless data as there exists a lack in the exactness of the results. Therefore, as a quality assessment, we analyzed the results of studies dealing with 95% confidence intervals smaller than 8 and control groups meeting HWE in a separate meta-analysis:

Summary meta-analysis -141C Del/Del (2)

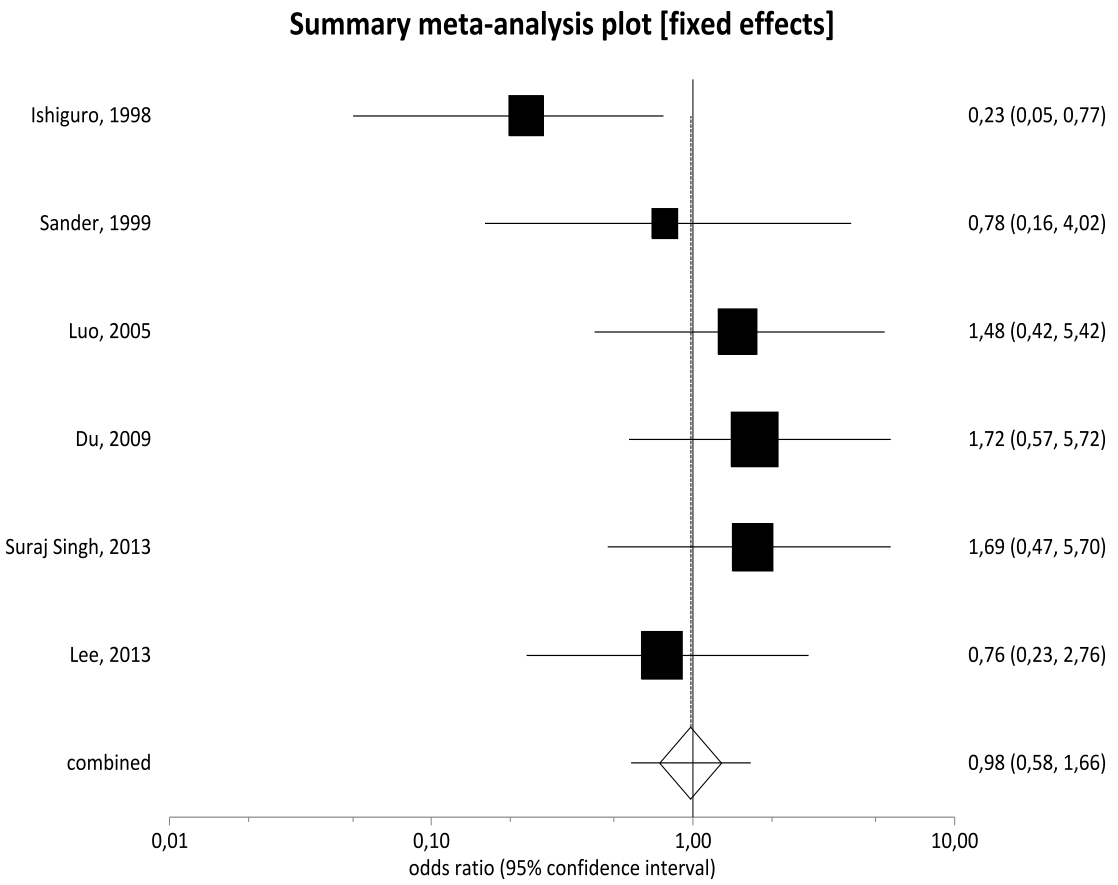


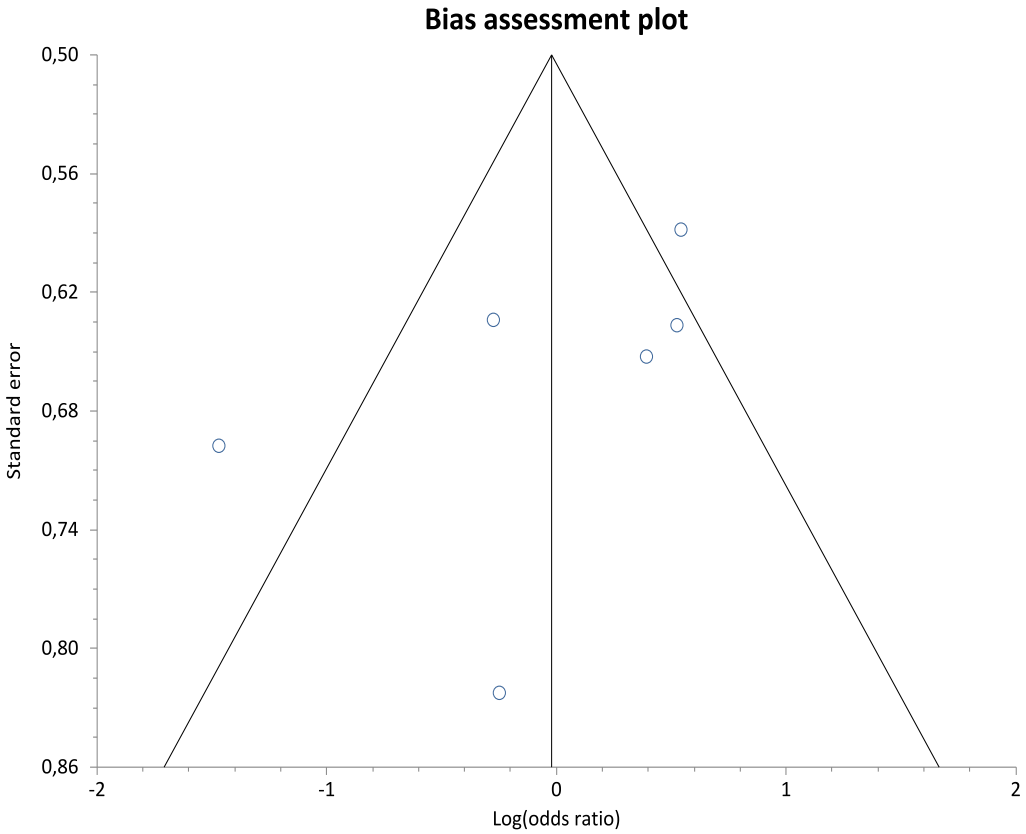
Figure 4.13
Association of the genotype -141C Del/Del of the promoter region of the DRD2 gene with alcohol dependence after leaving out the studies with too large 95% confidence intervals and the studies that didn't meet HWE in the control group. Forest plot showing ORs and 95% CIs for the studies (given by first author and year) included in the meta-analysis.

Results of the -141C Del/Del (2) meta-analysis: This meta-analysis implied absolutely no significantly altered risk for alcohol dependence by being carrier of the -141C Del/Del gene variant (OR=0,98; 95% CI=0,58-1,66) throughout any ethnicity examined. The individual odds ratios ranged from 0,23 to 1,72. The Cochran Q test (P=0,25) confirmed quite low between-study heterogeneity as well as the Inconsistency-test (I^2=24,3%). This led to the application of a fixed effects model (Mantel-Haenszel method).

There is completely no association between the -141C Del/Del genotype and an alteration in the risk of becoming alcohol dependent (Sander et al., 1999; Luo et al., 2005; Du et al., 2009; Suraj Singh et al., 2013; Lee et al., 2013). The majority of the studies doesn't give significant results thus emphasizing the -141C Del/Del genotype didn't play a role in the process of becoming alcohol dependent in the examined ethnic groups. After all there is no connection between the -141C genotypes and alcohol dependence in Asian and Caucasian populations found in our meta-analysis.

Discussion of the -141C Del/Del (2) meta-analysis: The influence of the SNP in the promoter region of the DRD2 gene on the receptor density (Ishiguro et al., 1998) doesn't appear to be clinically significant as it does not increase or decrease the likelihood of becoming alcohol dependent in any way. None of the examined ethnicities show any association between the -141C genotypes and alcoholism emphasizing that this SNP in all likelihood has no influence on the clinical drinking behaviour as well as on the overall dopamine receptor D2 density in the human brain. The dopamine metabolism which is involved in many central neuronal pathways and linked with psychiatric and addictive disorders, seems to play a role in alcohol dependence as well. Particularly the density of DRD2 expression is thought to be connected with the ethanol agency on the individual (Prasad et al., 2010). Therefore the genotypes of the TaqIA and -141C polymorphism of the DRD2 gene have been object of some case-control studies to differentiate between the genotypes and their drinking behaviour. While the TaqIA1 and the -141C Ins have been considered to cause a lower density of the DRD2 in the striatum, the nucleus accumbens and the amgdala (Lee et al., 2013) and hence have been considered to lead to a higher risk for alocohol dependence (Ishiguro et al., 1998), the TaqIA2 and -141C Del allele should then be responsible for a higher density of dopamine receptors D2 in these neuronal areas (Sander et al., 1999). In our meta-analysis we analyzed the influence of the homozygous genotypes on the likeliness of becoming alcohol dependent. We found significant association between the homozygous TaqIA genotypes and alcohol dependence in case of TaqIA1/1 being the high-risk genotype and also TaqIA1 being the polymorphism as it was available in lower frequencies among the studies' populations than the TaqI2 allele. We did not find any association between the polymorphisms of the examined gene sequence of the promoter region of the DRD2 gene (-141C) and alcohol dependence.

We were looking for publication bias in the following funnel plot:



4.14

The funnel plot is showing log(OR) and standard error for the association of -141C Del/Del with alcohol dependence after excluding several studies for the reasons mentioned above. The bias indicators showed no significant deviation from the symmetry assumption, thus not indicating publication bias. Egger's regression test: P=0,33.

Additionally a certain polymorphism in exon 8 of the dopamine receptor D2 gene has been examined for an association with alcoholism. Lee et al. (2013) and Luo et al. (2005) both investigated the impact of this exon 8 polymorphism on alcoholism. The study by Lee et al. (2013) gave numbers that confirm a positive association between the disease and the SNP considered. Delivering other results, the study by Luo et al. (2005) did not draw a linkage

between that SNP in exon 8 of the DRD2 and alcohol dependence. These controversial statements suggest that there is no clear association identified between the exon 8 polymorphisms and alcoholism yet. What is more the fact that there have not been enough studies investigating the importance of the polymorphism in exon 8 of the DRD2 gene left us not being able to include this genetic polymorphism into a meta-analysis but we collected the data given by the two studies in **Table 5.1** in the **Attachment**.

4) Discussion

As the ADH1 (ADH1A, ADH1B, ADH1C) is the isoenzyme of the alcohol dehydrogenase which has the biggest importance in ethanol degradation (Thomasson et al., 1991), it becomes obvious that the kinetics of this isoenzyme have a big impact on the time the human body is under the influence of alcohol. Especially two SNPs in ADH1B (adenine/guanine; Arg48His) and ADH1C (adenine/guanine; Ile350Val) have been taken into consideration to play a role in altering the risk of becoming alcohol dependent (Konishi et al., 2004). Our meta-analysis examined the meaning of being homozygous carrier of the alleles of these two SNPs. We found more than the double risk of becoming alcohol dependent when being carrier of the ADH1B*1/1 genotype. Thus we support several studies reporting that the ADH1B*1/1 represents the high-risk genotype as it leads to the integration of Arg which causes a slowly metabolizing isoenzyme (Aktas et al., 2012). The fact that this slow metabolism enlarges the time ethanol is circulating in the blood and leading to low central nervous alertness and activation of the reward system in the brain increases the risk of developing an alcohol addiction (Lu et al., 2001). We could support these results in any of the examined populations (Asian, Caucasian, European American, Mexican American). So compared with the heterozygous genotypes and the homozygous genotypes of the ADH1B*2 allele, carriers of the ADH1B*1/1 genotype are more likely to develop an alcohol addiction (Guo et al., 2010). On the other hand the ADH1B*2 allele leads to an isoenzyme that has a 100 times higher catalytic activity for ethanol oxidation than the ADH1B*1 isoenzyme (Yoshida et al., 1981). Hence the production of acetaldehyde works much faster and the time the body suffers from withdrawal symptoms is significantly enlarged (Huang et al., 2004). This makes regular alcohol consumption more unlikely as the time of reward feelings is only short compared with the time the carriers of the ADH1B*2/2 genotype have to cope with withdrawal symptoms (Choi et al., 2005). We calculated that ADH1B*2 homozygosity leads to only one third of the risk for becoming alcohol dependent in comparison with other genotypes of the ADH1B isoenzyme. Moreover we identified that Asian samples had much more participants being carrier of the ADH1B*2/2 genotype. Studies examining other ethnic groups didn’t even contain enough homozygous ADH1B*2 allele carriers to include them into

our meta-analysis. Consequently while the ADH1B*1/1 genotype is representing a high-risk genotype for the development of alcohol dependence throughout any ethnicity (Ehlers et al., 2012; Tóth et al., 2011; Guo et al., 2010), the ADH1B*2/2 genotype is protective at least in Asian populations for sure (Tan et al., 2010; Huang et al., 2004; Choi et al., 2005). Because this SNP is very rare in other ethnic groups, a protective effect towards alcoholism can only be assumed for these ethnicities (Cichoz-Lach et al., 2010). The ADH1C SNP (Ile350Val) has also been linked with having a connection with alcoholism (Kortunay et al., 2012). The ADH1C*2 (guanine) allele was considered to represent the high-risk allele, while the ADH1C*1 (adenine) allele should act protectively against alcohol dependence (Aktas et al., 2012). The mechanisms of interaction with the metabolism of ethanol remain the same as in the ADH1B polymorphisms. The ADH1C*1 allele encodes for a fast metabolizing isoenzyme which increases the amount of acetaldehyde leading to negative consequences after alcohol consumption, while the ADH1C*2 allele leads to a slowly metabolizing isoenzyme causing the fact that the individual benefits from drinking for a longer time, making regular alcohol intake more likely (Li et al., 2012). We analyzed the meaning of being homozygous carrier for these two alleles and did not find any association between the genotypes and an alteration in the risk of becoming alcohol dependent. So we supported studies that reported no association between the homozygous genotypes of the ADH1C gene with alcohol dependence (Ehlers et al., 2012; Tóth et al., 2011; Aktas et al., 2012). On the other hand there were some studies whose results were stating an association between the SNP and alcoholism (Konishi et al., 2004; Khan et al., 2010; Cichoz-Lach et al., 2010). But the direction of association of either the ADH1C*1/1 genotype or the ADH1C*2/2 genotype with alcohol dependence remained unclear in the end as there were different studies reporting different directions of association. This led to a combined effect that suggested the ADH1C homozygous genotypes having no influence at all on the clinical risk of becoming alcohol dependent. The alteration in the enzyme activity caused by the SNP that is supposed to have influence on the acetaldehyde level (Li et al., 2012) consequently is not clinically significant as there isn't any clear direction of association with alcohol dependence recognisable. Polymorphisms in other ADH isoenzymes have also been linked with alcohol dependence. In some studies the different genotypes of genes among the ADH4 gene were supposed to have an association with alcohol dependence (Edenberg et al., 2006; Luo et al., 2005; Preuss et al., 2011). In particular the studies by Luo et al. (2005) and Preuss et al. (2011) were considering

the two same genetic loci on chromosome 4q to be responsible for an altered risk of becoming alcohol dependent (rs1800759; rs1042364). Still there were no other studies referring to the meaning of these SNPs for the development of alcoholism which made it impossible to create a meta-analysis for the genotypes of these genes of the ADH4 gene. The same lack of sufficient data is on hand for other ADH isoenzymes such as the ADH2, ADH3, ADH6 or ADH7. Although some studies dealt with the importance of polymorphisms among the genes for alcohol dependence (Frank et al., 2012; Bucholz et al., 1994; Thomasson et al., 1991), there were not enough results given to be able to evaluate the clinical consequences of being carrier and especially homozygous carrier for one of the regarded SNPs. So we could perform our meta-analysis for the SNPs in the ADH1B and ADH1C genes only.

The acetaldehyde that is the product of the oxidation of ethanol needs to be degraded to avoid its accumulation which may lead to aversive reactions and cellular damage particularly in the brain (Hurley et al., 2012). The enzyme that is oxidizing acetaldehyde, the acetaldehyde dehydrogenase, is dividable into the most important ALDH1A, ALDH1B and ALDH2 which genetic information is located on chromosome 12q24.2-q24.12 (Zhang et al., 2014). In our meta-analysis we analyzed the meaning of being homozygous carrier of one of the ALDH2 alleles (guanine/adenine) compared with the other genotypes of this isoenzyme of the ALDH with the biggest importance for the catalytic activity (Hurley et al., 2012). The other isoenzymes are also located in the liver cells (Jackson et al., 2013) but they are not that important for the degradation of acetaldehyde and additionally thought to be only responsible for the oxidation of acetaldehyde when the ALDH2 is inactive (Hurley et al., 2012). The most examined single nucleotide polymorphism of the ALDH2 (guanine/adenine) leads to a switch from Glu to Lys at position 504 of the protein (Glu504Lys). The ALDH2*1 (Glu) allele is considered to lead to a fast-metabolizing isoenzyme, thus having a bringing forward effect on the development of alcoholism because it reduces the time the body is suffering from high acetaldehyde concentrations (Jo et al., 2007). The ALDH2*2 allele is causing the creation of a quite inactive ALDH2 isoenzyme which leads to the accumulation of acetaldehyde and the combined aversive reactions that make regular alcohol consumption more unlikely (Chen et al., 1999). We considered the homozygous carriers of these alleles to

be the best markers for the influence of the alleles on the drinking behaviour as they should reveal clear differences in drinking behaviour if the alteration in the enzyme activity is clinically significant. The majority of the studies we found dealing with the ALDH2 polymorphism and the risk of becoming alcohol dependent were examining Asian populations. Also the studies we could calculate ORs and 95% CIs for were dealing with Asian participants (Lee et al., 2001; Tan (Chin.) et al., 2010; Shin et al., 2010; Guo et al., 2010). The allele frequency of the ALDH2*2 allele was much higher in studies about Asian individuals than in studies analyzing another ethnic group which was also reported by other authors (Chen et al., 1999). Thus it was not possible to integrate studies about ethnicities different from Asian in our meta-analysis because they didn't contain enough ALDH2*2/2 genotypes (Konishi et al., 2004; Ehlers et al., 2012). Our meta-analysis didn't reveal a significant association between the ALDH2*2/2 genotype and alcoholism because we could only include four studies with quite large 95% confidence intervals due to the little number of ALDH2*2/2 individuals. Nevertheless we assume a protective impact of the ALDH2*2/2 genotype on the development of alcohol dependence because there were nearly no case subjects representing the ALDH2*2/2 genotype. Moreover the allele frequency of the ALDH2*2 allele was much lower in the case samples than in the controls. The comparably high number of ALDH2*2/2 carriers in Asian populations stresses that this ethnic group is protected from alcoholism by this SNP in the ALDH2 gene in contrast to other ethnicities such as European American, Native American, Caucasian (Amamoto et al., 2002). The wildtype allele of the ALDH2 gene, the ALDH2*1, is supposed to have a much higher efficiency than the one encoded by the ALDH2*2 allele (Tan et al., 2010). The ALDH1*1/1 genotype was found in a much higher frequency among all the studies. Our meta-analysis implied a significantly higher risk of becoming alcohol dependent by being homozygous carrier of the ALDH2*1 allele in comparison to the other genotypes of the ALDH2 gene. Thus we supported the findings of several studies reporting that the ALDH2*1/1 is a high-risk genotype for the development of alcohol addiction as the isoenzyme which is encoded by this genotype has a high catalytic activity and oxidizes acetaldehyde quickly (Chen et al., 1999; Chao et al., 2003; Tseng et al., 2007). So together with the findings of the importance of the ALDH2*1/1 genotype for alcoholism, it seems reasonable that the ALDH2*2/2 genotype is protected from alcohol dependence as several studies claimed (Jo et al., 2007) even if we could not stress this protective effect in a forest plot because this very rare genotype didn't allow us to

include a sufficient number of studies in the forest plot because there was an absolute lack of ALDH2*2/2 genotypes in particular among the case samples. However the increased risk of ALDH2*1/1 genotypes compared with the other allelic constellations suggests that one ALDH2*2 allele is enough for a clinically significant inactivation of the aldehyde dehydrogenase, thus representing the dominant allele (Matsuo et al., 2007). What is more there were some studies analyzing the meaning of ALDH1A and ALDH1B polymorphisms on the risk of becoming alcohol dependent. The ALDH1A1 allele has been considered with playing a role in the progress of alcohol dependence among Europeans (Lind et al., 2008). However there was not enough data given to analyze the regarded SNPs in a meta-analysis which might stress the statement that the meaning of the ALDH1A and ALDH1B SNPs on an alteration in the risk of becoming alcohol dependent is not clinically significant and much smaller than the meaning of the ALDH2 polymorphisms (Hurley et al., 2012).

The genetic information for the GABA-A receptor subunits that are also thought to have influence on the risk of becoming alcohol dependent are located on chromosome 4 (GABRA2, GABRA4, GABRB1 and GABRG1), on chromosome 5 (GABRA1, GABRA6, GABRB2, GABRG2) and on chromosome 15 (GABRA5, GABRB3 and GABRG3; Song et al., 2003). The best examined SNPs were all located among the GABRA2 gene. This subunit is supposed to be the major GABA-A receptor subunit in the limbic region (McKernan and Whiting, 1996) thus having big influence on the individuals' emotional reactions (Edenberg et al., 2004). It is already known as a target for anxiolytic medication (Rudolph et al., 1999). Ethanol causes emotional effects through GABAergic neurotransmission and consequently leads to an altered drinking behaviour that can be dependent on the genetics of the GABA-A receptor subunits, especially the α2-subunit (Täuber et al., 2003). Alcohol stimulates through complex mechanisms pre- and postsynaptic GABA receptors and leads in general to a decreased GABA-A receptor activation when progressing an addiction (Enoch, 2008). As there were not certain polymorphisms taken into consideration to play a role in altering the risk of becoming alcohol dependent as it was the case for ADH and ALDH genes, we analyzed four SNPs in the GABRA2 gene (rs279837, rs279844, rs279858, rs279869) that were the best examined in the studies we included in our meta-analysis. Only rs279858 (thymine/cytosine) lay among an

exonic region of the GABRA2 gene, all the other regarded sequences were introns (Lappalainen et al., 2005). We differed between the wildtype allele that was more common among the analyzed groups and the SNP and then analyzed the alteration in the risk for becoming alcohol dependent when being homozygous carrier for either the wildtype allele or the single nucleotide polymorphism. We calculated a significantly decreased risk of becoming alcohol dependent for the homozygous carriers of the wildtype alleles of the four regarded SNPs among the GABRA2 gene. The homozygous carriers of the single nucleotide polymorphisms were showing different results dependent on the SNP we're focussing on. The homozygous carriers of the polymorphisms in rs279837 and in rs279858 didn't show any association with an increased or decreased risk for becoming alcohol dependent during the homozygous carriers of the SNPs in rs279844 and in rs279869 had a slightly higher risk of becoming alcohol dependent caused by the genotype in comparison to heterozygous carriers of the polymorphism and homozygous carriers of the wildtype allele. The exact meaning of the polymorphisms in the subunits of the GABA-A receptor is still not completely described and thought to be very complex (Fehr et al., 2006). The reactions of the α2 subunit of the GABA-A receptor towards spontaneous and long-term alcohol consumption differ and particularly the long-term alcohol consumption which is responsible for developing addictions is not clarified completely. The polymorphisms that increase the risk of becoming alcohol dependent are considered to cause less receptor opening and thus less GABAergic transmission (Enoch, 2008) which leads to the problem of higher amounts of ethanol required to achieve emotional benefit from drinking. In which way the genetics influence the subunit expression seems to be complex (Edenberg et al., 2004) and is not understood completely yet. As our meta-analysis implies, being homozygous carrier of the wildtype alleles of the considered SNPs among the GABRA2 is protective against alcoholism. This leads to issue if already a heterozygous genotype increases the risk of becoming alcohol dependent in a specific manner as it alters the GABAergic neurotransmission in a clinically significant extent. So one mutated allele is probably enough to cause significantly decreased stimulation of the α2 subunit of the GABA-A receptor which leads to a higher amount of ethanol consumed to achieve benefit reactions from drinking mediated by GABA-A receptor openings. The α2 subunit is the best examined so far as it is located in a high amount in the limbic system thus being connected with addictions (McKernan and Whiting, 1996) and as it is already known for mediating the effects of anxiolytic medication (Rudolph et al., 1999).

Nevertheless other subunits of the pentameric ion channel have also been considered to have influence on the risk of becoming alcohol dependent. For example the GABRB1 polymorphisms have been linked with an alcohol addictive disease (Anstee et al., 2013; Parsian et al., 1997). Other studies reported weak linkage disequilibrium between GABRB1 variations and alcohol dependence (Song et al., 2003). Also other GABA-A receptor subunits have been taken into consideration to play a role in the development of alcoholism. Fehr et al. (2006) didn't only draw an association between the GABRA2 gene polymorphisms with alcohol dependence but also with SNPs in the GABRA6, GABRB2 and GABRG2 genes and the addiction. This again stresses the huge amount of subunits of the GABA-A receptors which makes it very difficult to analyze the influence of the genetics of GABA-A receptor subunits in the progress of alcoholism. In the end there is only evidence given for an association between specific genotypes among the GABRA2 gene and alcoholism. In particular the homozygous wildtype allele carriers seem to be protected from alcoholism in comparison to other genotypes in the respective genetic locations (Fehr et al., 2006). But still the exact mechanisms of interaction remain unknown (Edenberg et al., 2006). Also there is not one certain chromosomal area that is thought to influence the risk for alcohol dependence as it was the case with ADH and ALDH genes. Therefore it is necessary to clarify the role of the examined SNPs that are linked with alcohol dependence in further studies to increase the number of examined individuals. Hence we can differ between SNPs that are really altering the risk of becoming alcohol dependent in a significant manner and SNPs that don't alter the individual risk. Moreover the studies we analyzed were only dealing with Caucasian, Hispanic, European American and African American subjects. To investigate the inter-ethnic differences of the polymorphisms among the GABRA2 for the development of alcoholism, it will be necessary to analyze other ethnic groups, such as Asians, too.

The dopamine D2 receptor has also been linked with alcohol dependence (Chen et al., 2001). The genetic information of this receptor is located on chromosome 11q22-q23 (Prasad et al., 2010). The DRD2 is coupled with an inhibiting g-protein and it is mainly located in the striatum, the nucleus accumbens and the limbic system (Ishiguro et al., 1998). This emphasizes the role of the DRD2 in the neuronal reward system and the involvement in the

individual's emotions that both correlate with addictive behaviour as it is also the case for GABA-A receptor polymorphisms (Enoch et al., 2008). In contrast to the alterations in functioning of the GABA-A receptor subunits caused by genetic polymorphisms, the polymorphisms in the DRD2 gene don't lead to different opening activities of the dopamine D2 receptors but rather to different receptor densities (Lee et al., 2013). The SNP TaqIA in the ankyrin repeat and kinase domain containing one (ANKK1) gene is connected with an alteration in DRD2 density in the human brain (Gelernter et al., 1999). The ANKK1 gene is closely linked with the DRD2 gene on chromosome 11q23 thus having influence on the transcription rate (Lee et al., 2013). More exactly the ANKK1 gene lies 10 kb downstream of the DRD2 gene (Suraj Singh et al., 2013). In our meta-analysis we found a significant association between being homozygous carrier of the TaqIA1 allele (cytosine; 713Glu) and alcohol dependence and thus supported the theory of having a decreased amount of DRD2 receptors in the nucleus accumbens, the striatum and the mesolimbic system when representing the TaqIA1/1 genotype in comparison to other genotypes of the TaqIA (Cohen et al., 2007). This decreased amount of receptors requires a higher concentration of ethanol to achieve rewarding emotions through the dopamine pathway (Luo et al., 2005). This leads to a higher vulnerability of becoming alcohol dependent (Wang et al., 2007). On the other hand being homozygous carrier for the TaqIA2 allele (thymine; 713Lys) led to a significantly protective effect against alcoholism in our meta-analysis hence supporting findings that draw a connection between TaqIA2/2 genotypes and a higher amount of DRD2 receptors (Limosin et al., 2002). The direction of association of the homozygous genotypes of the TaqIA with alcohol dependence was the same particularly in Caucasians and Asians but also in other ethnic groups such as Mexican Americans for example. This emphasizes the meaning of the SNP in the TaqIA of the ANKK1 near the DRD2 gene for the development of alcohol dependence by altering the amount of DRD2 receptors (Cohen et al., 2007). Another SNP among the promoter region of the DRD2 gene has been linked with an altered risk of becoming alcohol dependent. An insertion or deletion of cytosine leads to -141C Ins or -141C Del in rs1799732. The insertion of cytosine is thought to cause a decreased DRD2 density, while the deletion of cytosine determines higher amount of dopamine D2 receptors (Arinami et al., 1997). As ethanol is known to boost the neuronal activity of dopaminergic neurons in the vetral tegmental area (Brodie et al., 1990) and stimulates the dopamine release in the nucleus accumbens (Weiss et al., 1993), the receptor density is of outstanding

importance for the cellular reaction towards alcohol consumption. The TaqIA polymorphisms already showed off how important the heritable receptor amount can be for the risk of becoming alcohol dependent. The -141C Ins/Ins genotype has also been recognized to be the high-risk genotype for alcohol dependence by Ishiguro et al. (1998) and Lee et al. (2013). The -141C Del/Del genotype however was connected with a significant protection from alcoholism as this genotype leads to an increased amount of dopamine D2 receptors (Ishiguro et al., 1998; Prasad et al., 2010). In contrast to that several studies reported no association between the different -141C genotypes and alcohol dependence (Sander et al., 1999; Du et al., 2009; Luo et al., 2005; Suraj Singh et al., 2013). Consequently we didn't find any association between the homozygous carriers for the -141C Ins or the -141C Del alleles (Cys311Ser) and the risk of becoming alcohol dependent in our meta-analysis. So the influence of the polymorphism on the receptor density is not clinically significant as it doesn't alter the overall drinking amount (Sander et al., 1999). There are too many opposite effects on the drinking behaviour for both homozygous genotypes reported, for example the -141C Ins/Ins genotype is associated with a protection from alcoholism by some authors (Suraj Singh et al., 2013; Chen et al., 2001; Gelernter et al., 1999). So there doesn't exist a clear direction of association between the SNP in the promoter region -141C of the DRD2 gene and alcohol dependence throughout any ethnicity in contrast to the polymorphisms of the TaqIA. Exon 8 has also been connected with alcohol dependence by Lee et al. (2013) and Luo et al. (2005). The studies however gave controversial results and were the only two studies that were investigating the meaning of polymorphisms among exon 8 of the DRD2 and a variation in the risk of becoming alcohol dependent hence not being useful for a statistical analysis.

5) Conclusion

The development of alcohol dependence is thought to be dependent on both environmental and genetic factors (Bosron et al., 1989). In our meta-analysis we focused on the genetic polymorphisms in ADH, ALDH, α2 subunit of the GABA-A receptor as well as TaqIA and -141C of the DRD2 genes for a variation in the risk of becoming alcohol dependent when being homozygous carrier of one of the regarded alleles. By analyzing homozygous carriers we hoped to achieve clearer results as for heterozygous carriers for who the genetic effect of the SNP should not be as significant theoretically. A problem consisted in the fact that homozygousity can be very rare for some alleles, for example the ALDH2*2 allele. This makes a calculation of odds ratios quite difficult. But on the other hand if we counted enough homozygous carriers of the regarded allele, we could achieve significant results for some polymorphisms. The homozygous carriers of the ADH1B alleles, the ALDH2*1 allele, the TaqIA alleles and at least the homozygous wildtype allele carriers of the regarded genetic sequences among the GABRA2 gene showed significant association with alcohol dependence. On the other hand being homozygous carrier of the ADH1C alleles, the ALDH2*2 allele, the -141C alleles and some SNPs of the GABRA2 didn't show any association with alcoholism in our meta-analysis. So after all we could illustrate two aspects. First genetic predispositions cause immense alterations in the individual risk of becoming alcohol dependent regardless from the environmental factors (Edenberg et al., 2006). By regarding the genotypes one can make a statement about the likeliness of becoming alcohol dependent even before the first alcohol intake. It is important to process prevention programs in particular for the high-risk genotypes. Hence one can at least try to prohibit family-cumulative alcohol dependence. Second even if animal experiments report an alteration in ethanol impact caused by differences in the genetic background, the meaning of the polymorphism for the individual overall risk of becoming alcohol dependent is not assured at all. For example animal experiment were showing a significant meaning of -141C polymorphisms on the dopaminergic neurotransmission (Weiss et al., 1993) but case-control and cohort studies didn't find an association between the genotypes and alcohol dependence. This again emphasizes the importance of case-control and cohort studies to

identify genotypic risk constellations. Animal experiments can explain pathophysiological mechanisms but case-control studies are needed to depict the clinical relevancy of the genetic polymorphisms. Alcohol dependence is a disease that is of outstanding importance for clinical occupation. This is why it is essential to get to know more about possible genetic risk factors as a prediction marker and as well as a potential therapy strategy in the future. Our meta-analysis outlines the meaning of being homozygous carrier of polymorphisms of the ADH, ALDH, GABRA2 and DRD2 genes influencing the individual risk of becoming alcohol addicted but it also stresses the fact that there is further research necessary to glean each of the single nucleotide polymorphisms in the genes that have influence on the development of alcohol dependence.

6) Attachment

6.1) Rare polymorphisms associated with AD

We also found some rare SNPs that were considered by some studies to have an influence on the likeliness of becoming alcohol dependent. As the number of information given was too small to include them in a statistical analysis we collected the regarded polymorphisms and summed up the studies' characteristics in the following table keeping in mind that the SNPs could be connected with a clinically higher risk of becoming alcohol dependent such as some of the well-studied polymorphisms above but also that there is no sufficient data available to give evidence for an existing significant association with alcohol dependence.

SNPs[a]	Genetic locus[b]	Author	Year	n (cases)[c]	n (controls)[c]
ADH2	Chromosome 4 exon 3, exon 9	Thomasson et al.	1991	49	47
ADH3	Chromosome 4 exon 8	Thomasson et al.	1991	49	47
ADH4	Chromosome 4 rs1042363, rs1800759	Luo et al.	2005	560	365
ADH4	Chromosome 4 -192bp, -159bp, -75bp	Guindalini et al.	2005	92	92
ADH4	Chromosome 4	Edenberg et al.	2006	COGA	-

	rs4148886			sample	
ADH4	Chromosome 4 rs1042364, rs1800759	Preuss et al.	2011	1622	1469
ADH7	Chromosome 4 rs284779	Edenberg et al.	2006	COGA samples	-
ADH6	Chromosome 4	Zuo et al.	2013	3723	5948
ADH7	Chromosome 4	Zuo et al.	2013	3723	5948
ALDH1A	Chromosome 9 rs348449	Lind et al.	2008	104	201
GABRB1	Chromosome 4 (tetranucleotide polymorphisms)	Parsian et al.	1997	133	89
GABRA5	Chromosome 15	Song et al.	2003	COGA sample	-
GABRB1	Chromosome 4 (tetranucleotide polymorphisms)	Song et al.	2003	COGA sample	-
GABRB3	Chromosome 15	Song et al.	2003	COGA sample	-
GABRB2	Chromosome 5 rs3780428	Terranova et al.	2013	186	139
DRD2	Chromosome 11 rs6276	Luo et al.	2005	200	251
DRD2	Chromosome 11 rs6276	Lee et al.	2013	189	110

[a] regarded SNPs in the respective

[b] exact genetic locus of the regarded SNP if information provided by the study

[c] total number of cases and controls in the relative study

Table 5.1

Characteristics of the studies dealing with rare polymorphisms that are associated with alcoholism given by the regarded SNP, the exact genetic locus, first author, year of publication, total number of cases as well as total number of controls.

6.2) GABA-A receptor genes

We listed the studies about the influence of GABRA2 gene polymorphisms on the risk of alcohol dependence in another order, this time focusing the year of publication to be able to evaluate if differences in association between the SNPs and alcoholism are dependent on the time the study was processed. In so doing we could not just examine the meaning of a certain SNP among the GABRA2 gene for the development of alcohol dependence but also if the outcome of the studies is influenced by the individual study design as well as the time the analysis was performed, thus indicating that not just the genetics play a role in the issue of GABRA2 genes altering the risk for alcoholism.

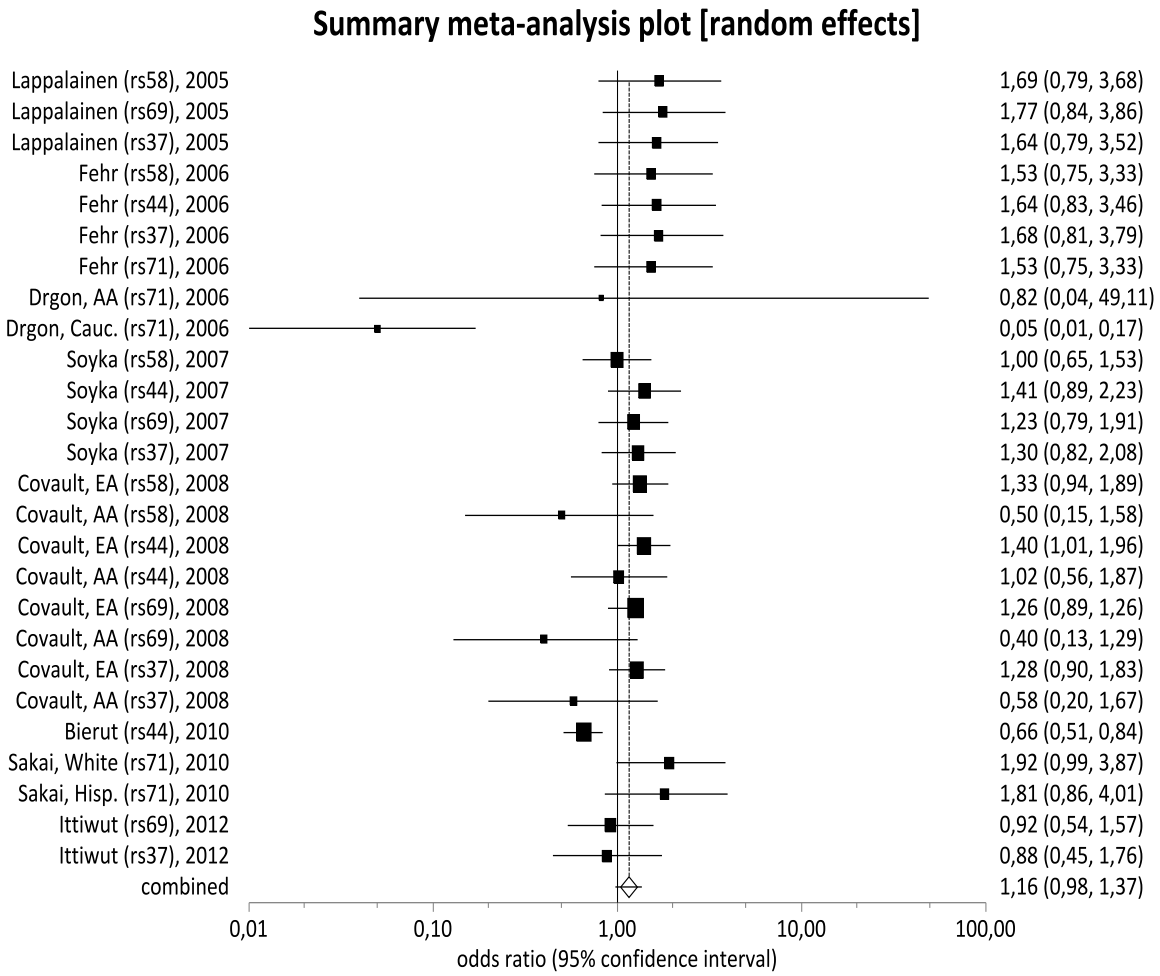


Figure 5.1
Association of the homozygous carriers of the GABRA2 SNPs with alcohol dependence. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the

GABRA2 gene and year) included in the meta-analysis (Hisp.=Hispanic, EA=European American, AA=African American).

Results of the GABRA2 SNPs meta-analysis: There is no significant alteration in association of the regarded SNPs among the GABRA2 gene with alcoholism by regarding the year the respective study was processed. So the time the participants were examined for their genetic risk on developing alcohol dependence doesn't play a role for the studies' outcome. Also there are no significant differences between the studies hence we cannot consider an alteration of the results by differences in the study designs.

We investigated the meaning of the time the study was carried out on the risk of alcohol dependence for homozygous wildtype allele carriers in the following forest plot after we processed our sensitivity analysis and quality assessment:

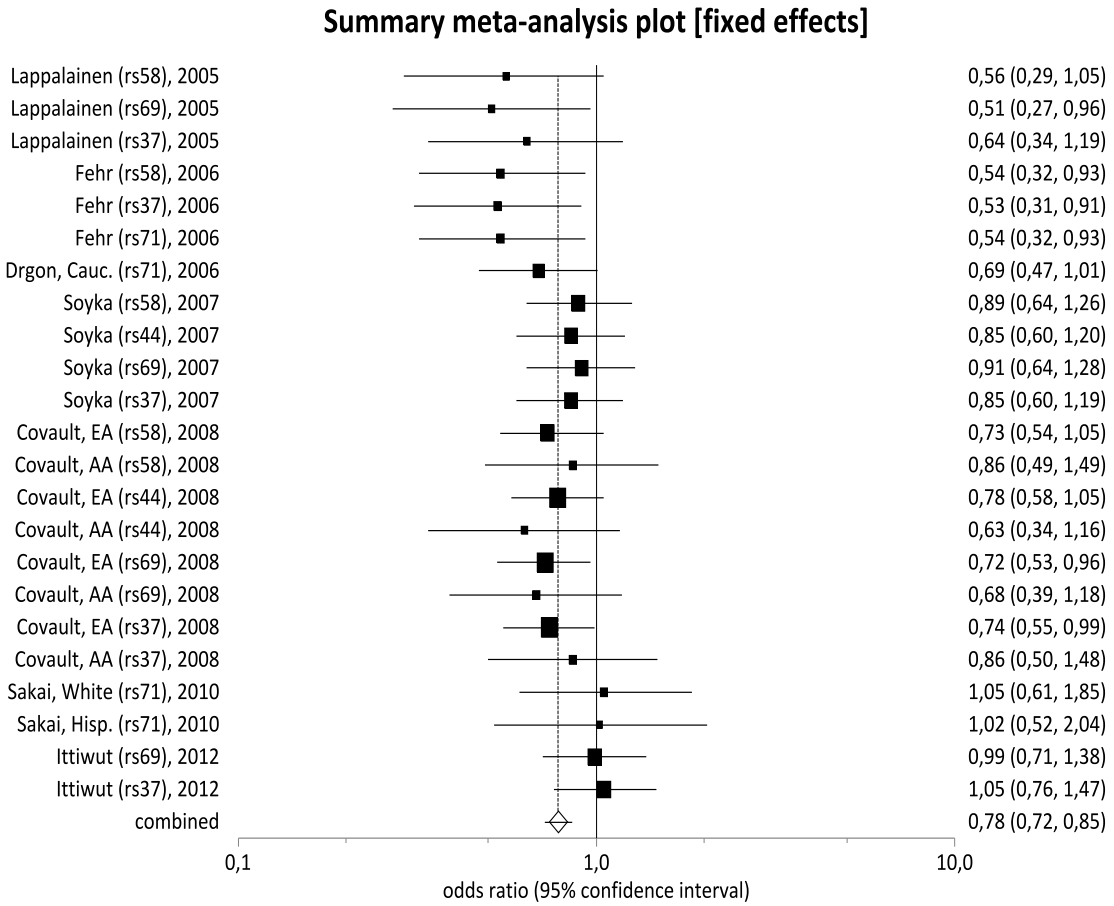


Figure 5.2
Association of the homozygous carriers of the GABRA2 wildtype alleles with alcohol dependence after excluding studies without the control samples meeting HWE and studies with 95% CIs larger than 8. Forest plot showing ORs and 95% CIs for the studies (given by first author, SNP rs(2798)xx in the GABRA2 gene and year) included in the meta-analysis (Hisp.=Hispanic, EA=European American, AA=African American).

Results of the GABRA2 wildtype allele meta-analysis: This figure reveals that the latest studies we included in our analysis were showing no impact of homozygosity in GABRA2 wildtype alleles on the risk of becoming alcohol dependent. Some earlier studies reported a statistically significant protection by being homozygous carrier of the GABRA2 wildtype alleles (Fehr et al., 2006; Lappalainen et al., 2005). However the majority of the studies didn't report a significant association. This is why there is no evidence given for regarding the time of the studies' publication to be a factor that has a statistically significant impact on the outcome. We still consider the homozygous genetic polymorphsims being responsible for the

alteration in the risk of becoming alcohol dependent. The combined effect shows that there is a siginifantly negative association between the homozygous genotypes of the wildtype alleles in the GABRA2 gene and the risk of becoming alcohol dependent.

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