KUOPION YLIOPISTON JULKAISUJA D. LÄÄKETIEDE 465
KUOPIO UNIVERSITY PUBLICATIONS D. MEDICAL SCIENCES 465

TERO HALLIKAINEN

Serotonin and Dopamine Gene Polymorphisms and Alcohol Consumption

Serotoniinin ja dopamiinin aiheenvaihduntaa koodaavien geenien vaikutus alkoholin kulutukseen

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium ML2, Medistudia building, University of Kuopio, on Friday 4th December 2009, at 12 noon

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KUOPION YLIOPISTO

KUOPIO 2009

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ISBN 978-951-27-1365-3
ISBN 978-951-27-1382-0 (PDF)
ISSN 1235-0303

Kopijyvä
Kuopio 2009
Finland

Hallikainen, Tero. Serotonin and Dopamine Gene Polymorphisms and Alcohol Consumption. Kuopio University Publications D. Medical Sciences 465, 2009. 150 p.
ISBN 978-951-27-1365-3
ISBN 978-951-27-1382-0 (PDF)
ISSN 1235-0303

ABSTRACT

It is common knowledge and has been shown in studies that alcoholism clusters in families. However, the model of inheritance in vulnerability to alcoholism is believed to be complex and polygenic, different subtypes of the disorder probably having different genetic aetiologies with a varying environmental impact on the development. Already in the 1980s C.R. Cloninger proposed his dichotomy of two subtypes in alcoholism: late-onset, socially dependent type 1, and early-onset, antisocial, impulsive violent type 2. The genes involved in serotonin and dopamine neurotransmission of the brain have been extensively studied, because these transmitters are believed to be crucial in mediating the acute effects of alcohol. However, the results of these studies have been equivocal, as alcoholics have most often been studied as one group suffering from a homogenous disorder.

Here, alcoholic subjects were classified as type 1 or type 2, and the COMT (coding cerebral dopamine inactivation) and 5-HTTLPR (coding serotonin transporter synthesis) genotypes thereafter compared between these groups and healthy controls (and the general population) in studies I–III. In studies IV–V, COMT and (DRD2) TaqI A (possibly affecting dopamine DRD2 receptor availability in the human brain) genotypes were studied among a large sample of non-alcoholic socially drinking males.

Type 1 alcoholism (including the majority of alcoholics) in these studies showed an association with dopaminergic polymorphism COMT (L allele), but not with serotonergic 5-HTTLPR. Type 2 alcoholism (with ASP and habitual impulsive violent behaviour) showed an association with serotonergic polymorphism 5-HTTLPR (S allele), but not with dopaminergic COMT. The results are consistent with the dichotomy of dopaminergic and serotonergic deficits in these subtypes of alcoholism, previously suggested by Cloninger before the era of molecular genetics. Dopaminergic polymorphisms COMT and TaqI A showed an association with alcohol consumption among socially drinking males, indicating the role of dopamine-mediated reward mechanisms in regulating non-alcoholic drinking patterns, as well. Careful diagnostic procedures, classification and subtyping of alcoholic study subjects are essential considering the heterogenous nature of this disorder. Comorbid disorders should be screened for to support the formulation of clinical subtypes. This is crucial concerning both research and individual treatment planning.

National Library of Medicine Classification: QV 126, QV 76.5, WK 725, WL 102.8, WM 274
Medical Subject Headings: Alcohol Drinking/genetics; Alcohol Drinking/metabolism; Alcoholism/genetics; Dopamine/metabolism; Finland; Humans; Serotonin/metabolism

Hallikainen, Tero. Serotoniinin ja dopamiinin aiheenvaihduntaa koodaavien geenien vaikutus alkoholin kulutukseen. Kuopion yliopiston julkaisuja D. Lääketiede 465. 2009. 150 s.
ISBN 978-951-27-1365-3
ISBN 978-951-27-1382-0 (PDF)
ISSN 1235-0303

TIIVISTELMÄ

Perinteisesti on tiedetty, ja tutkimukset ovat sen myöhemmin osoittaneet, että alkoholismia esiintyy suvuittain. Riski sairastua alkoholismiin periytyy, mutta on polygeenisesti (useiden geenien ja näiden yhdistelmien välityksellä) säädelty. Ympäristövaikutuksilla on vaihteleva osuus häiriön synnyssä, riippuen myös alkoholismin alatyypistä. Jo 1980-luvulla C.R. Cloninger ehdotti alkoholismin jakoa kahteen alatyyppiin: myöhemmällä iällä alkavaan sosiaaliseen tyyppi 1:een ja varhain alkavaan antisosiaaliseen tyyppi 2:een, jossa esiintyy impulsiivista väkivaltaisuutta. Aivojen välittäjäaineista serotoniinin ja dopamiinin on uskottu olevan keskeisiä alkoholin akuutin keskushermostovaikutuksen ja nousuhumalaan liittyvän mielihyvän aiheuttajina. Näiden välittäjäaineiden aineenvaihduntaa säätelevien geenien osuutta alkoholismialttiuteen on tutkittu paljon. Tulokset ovat kuitenkin olleet ristiriitaisia, kun alkoholisteja on useimmiten tutkittu yhtenäisenä ryhmänä, luokittelematta eri alatyyppeihin.

Näissä tutkimuksissa alkoholismista kärsivät tutkimushenkilöt on luokiteltu tyyppi 1- ja tyyppi 2-alkoholisteiksi. COMT- ja 5-HTTLPR-genotyyppejä verrattiin näiden kahden alkoholistiryhmän, terveiden kontrollihenkilöiden (ja yleisväestön) välillä tutkimuksissa I–III. COMT-geeni koodaa entsyymiä, joka säätelee dopamiinin inaktivaatiota aivoissa, ja 5-HTTLPR-geeni koodaa serotoniinitransportterin synteesiä. Tutkimuksissa IV–V selvitettiin suuressa joukossa sosiaalisesti juovia miehiä, vaikuttaako COMT- tai (DRD2) TaqI A–genotyyppi alkoholin kulutukseen. TaqI A-geeni säätelee mahdollisesti aivojen dopamiinin aineenvaihduntaa.

Tyyppi 1-alkoholisteilla havaittiin assosiaatio dopaminergisen COMT-polymorfismin L-alleeliin mutta ei assosiaatiota serotonergiseen 5-HTTLPR-genotyyppiin. Tyyppi 2-alkoholisteilla puolestaan havaittiin assosiaatio 5-HTTLPR-polymorfismin S-alleelin kanssa mutta ei COMT-polymorfismin kanssa. Löydökset tukevat Cloningerin aiempaa oletusta näiden alkoholismin alatyyppien taustalta löytyvästä dopaminergisestä (tyyppi 1) tai serotonergisestä häiriöstä (tyyppi 2). Dopaminergisillä polymorfismeilla COMT- ja Taq1 A osoitettiin assosiaatio sosiaalisesti alkoholia käyttävien miesten viikoittain käyttämiin alkoholimääriin. Tämä tuki oletusta, että dopamiiniaiheenvaihdunnalla on merkitystä juomatapojen säätelijänä silloinkin, kun kyseessä ei ole alkoholismi. Alkoholismia tutkittaessa huolellinen diagnostiikka ja jako alatyyppeihin on keskeistä, kun kyseessä mitä ilmeisemmin näidenkin tutkimusten valossa on heterogeeninen, epäyhtenäinen joukko sairastuneita. Samanaikaiset psyykkiset häiriöt tulisi diagnosoida ja seuloa alkoholismin alatyyppien tunnistamiseksi (mm. tyyppi-2-alkoholismiin liittyvä antisosiaalinen persoonallisuushäiriö). Tämä on olennaista sekä tutkimuksen että yksilöllisen hoidon suunnittelun kannalta.

Yleinen suomalainen asiasanasto: aineenvaihdunta; alkoholismi; dopamiini; juomatavat; perinnöllisyys; serotoniini; välittäjäaineet

This work is dedicated to all those wondering
whether addiction is a disease or not,
and to those who are not sure if they have been affected or not.

ACKNOWLEDGEMENTS

This thesis is based on the research in the Department of Forensic Psychiatry, University of Kuopio, Niuvanniemi Hospital, and in the Department of Public Health, University of Kuopio during the years 1998-2009. It should be best considered as a piece in the puzzle of the long-term research activity in the Department of Forensic Psychiatry, launched in the 1980s to study the aetiology of violence and substance use disorders. The work on this thesis was financially supported by the Department of Forensic Psychiatry and Panu Hakola's Fund (Panu Hakolan Rahasto).

My deepest and humble gratitude goes to my first supervisor, Professor Jari Tiihonen for proposing this line of study, actually inventing the topics, and helping and guiding me all the way from the very beginning to the revising and finishing of this thesis. I even began to wonder if his patience never wears out as the years passed and there was not a visible sign of a literature review. Since the thesis quite unexpectedly is finished I am obliged to sincerely thank Professor Tiihonen for this obligatory learning experience: when I started I knew hardly anything about genetics, and certainly nothing about molecular genetics. This thesis owes its existence to professor Jari Tiihonen.

I express my deep gratitude to my second supervisor Professor Jussi Kauhanen, as well. He has been a dedicated guide and an essential collaborator in all studies, alone in recruiting the study subjects. Professor Kauhanen's role was crucial in every aspect in studies IV and V, and in the revising the manuscript of the thesis. I thank him for his patience as well.

I wish to thank warmly the official referees of this thesis, Professor Matti Virkkunen, and Docent Hannu Naukkarinen, for their valuable and constructive criticism and advice in improving the manuscript. In particular, I need to thank Professor Virkkunen for encouragement and counselling in the writing process through these years. I could nothing but admire his prompt way of revising the manuscript among his numerous other duties. Professor Virkkunen earns many credits for the up-to-date form of the thesis.

With all my heart I want to thank Tarja Koskela and Aija Räsänen for their secretarial assistance through these 11 years, at first with my hand-written manuscripts of the original publications, ending up with organizing the public examination of the printed thesis. You have both been of indispensable aid. Without Tarja's efforts and professional editorial skills this thesis would still be a pile of hand-written notes.

I wish to thank all my co-authors for the work involved in the original publications. Vivian Michael Paganuzzi revised the language of the manuscript (except this section), and I am most grateful for that. Even before this he tried to teach me that knowing some scientific jargon or pop song lyrics is different from knowing the English language. Here, I also want to thank all those study subjects and controls, unknown to me, who have volunteered to give their blood samples for DNA extraction to help the medical science.

Emeritus Professor Panu Hakola, the former Medical Director of Niuvanniemi Hospital has to be credited for my education and the choice of career as forensic psychiatrist. He was my first and the most impressive teacher in clinical psychiatry and forensic psychiatry. As the founder of the Department of Forensic Psychiatry and the research activity in Niuvanniemi Hospital he has always emphasized the connection between clinical practise and research. With these cordial thanks I wish him many active retirement years still.

I am deeply grateful to Docent Eila Tiihonen, the Medical Director of Niuvanniemi Hospital in these years that I've spent working, and not working, on this thesis. She has supported me to carry on finishing the manuscript, and her administrative skills have ensured me time and space to work on it. Docent Eila Tiihonen as my immediate superior has been a tutor beyond compare in clinical forensic psychiatry as well. I also want to thank my colleagues in the hospital for your flexibility concerning my research duties. However, I am not quite sure if it was your win or my gain to keep me out of the clinical practise every now and then. My special thanks go for the colleagues who have defended their thesis in public before me: Markku Eronen, late Erkki Tupala, Jarmo Paanila, and Anu Putkonen. You have shown me the way.

I owe my mother more than I will be able to give back, for bringing me up and supporting through my life in her quiet, modest way. I am grateful to the rest of my family, as well. My father did not live old enough to see this thesis ready. Thinking of him now I can imagine his comments: “This is all very well from you, son. I wouldn’t know better myself. But if I remember right, you were not in the trenches during the World War II, were you.” I’ve begun to understand that my father was right in many things. The trenches and minefields remind me of my long-term friends Vesa and Veli-Matti. One of them supported me occasionally by telling that a thesis very seldom gets ready on its own, without the author touching it. The other one told me to mind my own business because he was hungry or sleepy. With these clear instructions finishing the thesis did not take too long, thank you for your contribution.

To my dearest wife Pauliina: thank you for giving me the occasional peace of mind, constant one is out of my reach. I have appreciated, more than you would imagine, your laconic way of creating an atmosphere of no pressure during this last year’s work. You don’t need Gerald and Higley’s (2002) rhesus monkeys to tell you that it is not a piece of nuts and raisins to live with an aging genetically unrelated male, even if he would have the fine polished traits of primate nature, such as mine: long-term unforgiving hatred with unforeseen bursts of anger. I believe nobody could have done it better than you to survive. You even managed to keep the youngsters quiet. I want to give my sincere loving gratitude to you, Pauliina. Oona and Simeon, you can speak up now, the year has passed.

Finally, I want to thank my children Mikael and Maria, for just being who you are. Your existence has helped me through my deepest moments of despair. You’ve learned in practise the power of behavioral genetics: being psychologically very similar to another person makes it sometimes hard to be together, but also difficult to be far apart. Just between us: you are my popsicle because I wanted to have it.

Kuopio, November 2009
Tero Hallikainen

Abbreviations

5-HIAA	5-hydroxyindolacetic acid
5-HT	5-hydroxytryptamine, serotonin
5-HTT	serotonin transporter (SERT)
5-HTTLPR	5-HTT gene linked polymorphic region
AD	alcohol dependence
ADH	alcohol dehydrogenase
ADHD	attention deficit hyperactivity disorder
ALDH	aldehyde dehydrogenase
APA	American Psychiatric Association
ASP, ASPD	antisocial personality disorder
BP	binding potential
Caucasian	white ethnic group
CNS	central nervous system
CNV	copy number variation
COGA	Collaborative Study on the Genetics of Alcoholism
COMBINE Study	Combined Pharmacoterapies and Behavioral Interventions for Alcohol Dependence Study
COMT	catechol-O-methyltransferase
CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
DA	dopamine
DAT	dopamine transporter
DNA	deoxyribonucleic acid
DRD2	dopamine receptor type D2
DSM-III-R, -IV	Diagnostic and Statistical Manual of Mental Disorders
ECA	epidemiological catchment area
ERP	event-related potential
ethanol	alcohol
F-N Study	Finn-NIAAA study project of violent alcoholics
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GxE	gene-environment (interaction)
GGT	gamma-glutamyltranspeptidase
GMR	glucose metabolic rate
GWS	genome-wide scan
haplotype	alleles located at nearby, linked loci
HHRR	haplotype relative risk calculation
HPA	hypothalamus-pituitary-adrenal cortex
H-W	Hardy-Weinberg (equilibrium)
ICD	International Classification of Disease
KIHD	Kuopio Ischemic Heart Disease Risk Factor Study
knock-out	laboratory animal with a particular gene disrupted
LD	linkage disequilibrium
LR	level of response
MAO	monoamine oxidase

MAST	Michigan Alcoholism Screening Test
MCV	mean corpuscular volume (of red blood cells)
MD	major depression
MJD	marijuana dependence
MRI	magnetic resonance imaging
NAC	nucleus accumbens
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NMDA	N-methyl-D-aspartate receptor of glutamate
NPY	neuropeptide Y
OCD	obsessive-compulsive disorder
OR	odds ratio
PCR	polymerase chain reaction
PET	positron emission tomography
PTSD	post-traumatic stress disorder
RFLP	restriction fragment length polymorphism
RR	risk ratio
SD	standard deviation
SERT	serotonin transporter (5-HTT)
SNP	single nucleotide polymorphism
TDT	transmission disequilibrium test
TPQ	Tridimensional Personality Questionnaire
VNTR	variable number tandem repeat
VTA	ventral tegmental area
WGA	whole genome association
WHA	whole hemisphere autoradiography

List of original publications

This thesis is based on the following original publications:

I Tiihonen J, Hallikainen T, Lachman H, Saito T, Volavka J, Kauhanen J, Salonen JT, Ryynänen O-P, Koulu M, Karvonen MK, Pohjalainen T, Syvälahti E, Hietala J. Association between the functional variant of the catechol-O-methyltransferase (COMT) gene and type 1 alcoholism. Molecular Psychiatry 1999;4:286–89.

II Hallikainen T, Lachman H, Saito T, Volavka J, Kauhanen J, Salonen JT, Ryynänen O-P, Koulu M, Karvonen MK, Pohjalainen T, Syvälahti E, Hietala J and Tiihonen J. Lack of association between the functional variant of the catechol-O-methyltransferase (COMT) gene and early-onset alcoholism associated with severe antisocial behavior. American Journal of Medical Genetics (Neuropsychiatric Genetics) 2000;96:348–352.

III Hallikainen T, Saito T, Lachman HM, Volavka J, Pohjalainen T, Ryynänen O-P, Kauhanen J, Syvälahti E, Hietala J and Tiihonen J. Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. Molecular Psychiatry 1999;4:385–388.

IV Kauhanen J, Hallikainen T, Tuomainen T-P, Koulu M, Karvonen MK, Salonen JT and Tiihonen J. Association between the functional polymorphism of catechol-O-methyltransferase gene and alcohol consumption among social drinkers. Alcoholism, Clinical and Experimental Research 2000;24:135–39.

V Hallikainen T, Hietala J, Kauhanen J, Pohjalainen T, Syvälahti E, Salonen JT and Tiihonen J. Ethanol consumption and DRD2 gene TaqI A polymorphism among socially drinking males. American Journal of Medical Genetics 2003;119A:152–155.

Contents

1 INTRODUCTION

In 2006, 88 % of the adult population in Finland reported using alcohol. The number of heavy consumers is estimated to be 6–12 % of the adult population, as many as 500 000 individuals. This 10% of drinkers with the highest consumption account for almost half of the alcohol (for over 40%) consumed in Finland. Most young people start to experiment with drinking between the ages of 12 and 16 years, and only one-fifth of 16-year-olds were not using alcohol at all in 2006. The latest rapid increase in consumption and adverse effects of the increase were seen after the lowering of taxes on alcoholic beverages in the beginning of 2004. In 2006, the average adult total alcohol consumption in Finland was 10.6 litres of 100% alcohol. The total consumption has more than doubled over the past three decades. This has brought Finland to the medium level of alcohol consumption among European countries. Compared with them, binge drinking (heavy intoxication) still continues to be more common in Finland (Mäkelä, 2003; Nylander et al., 2007).

The number of alcohol-related deaths was estimated as 3050 in 2006, and there was an increase of over 20% in the seven years since 1999, and an especially rapid growth since 2004. The increase was exclusively due to alcohol-related diseases or alcohol poisonings, since the number of fatal accidents or violence with alcohol involved had not changed. Alcohol-related causes were the most common cause of death among working-age (15–64 years) males and females in Finland in 2006. In recent years, some 80% of the fatal violent offences and 70% of assaults have been committed under the influence of alcohol. It was estimated that in 2005 Finnish society paid almost one million euros in direct costs, and between three and six billion euros in indirect costs, as a result of alcohol use (Nylander et al., 2007).

The clinical outcome of alcoholism after a standard detoxification treatment is known to be poor, even if psychosocial therapy is implemented, up to 70% of alcoholics resuming drinking within one year. However, the results of the research in the past 30 years suggest that individuals who had obtained

treatment for their drinking problem still had a slightly better outcome than those who did not (Johnson, 2008). Even so, the data from long-term follow-up studies suggest a very low effectiveness of the treatments available. In addition, several large studies have shown that the traditional Twelve Step Facilitation of Alcoholics Anonymous continues to be as effective as the more modern and costly cognitive and behavioural therapies (Room et al., 2005). The medications tried so far have shown only a modest additive effect, and the efficacy is usually seen only in limited subpopulations of the treated alcoholics. Molecular genetic studies are expected to help in targeting the existing pharmacological treatments at different types of alcoholics for a better response, and to develop completely new treatments by detecting the differences in neurobiology of the various forms of alcoholism (Room et al., 2005; Johnson, 2008).

The individual vulnerability to alcohol use disorders can be both inherited and environmental. Family studies have shown this to be true, and twin pair studies have suggested that genetic and environmental factors are equally important in determining the risk for alcoholism (50-50%). Numerous adoption studies have also provided strong support for the action of genetic factors (Goldman et al., 2005a). Based on the strong familial aggregation of alcohol abuse and twin studies in Sweden and in the USA, C.R. Cloninger proposed the dichotomy of two subtypes in alcoholism: late-onset, socially dependent, cautious type 1, and early-onset, antisocial, impulsive violent type 2. Cloninger originally proposed a dichotomy of dopaminergic and serotonergic deficits in neurotransmission between these subtypes (Cloninger, 1987; Cloninger, 1995). Later studies on the neurobiology of alcoholism and violence have supported Cloninger's ideas (Bowirrat and Oscar-Berman, 2005). In the field of molecular genetics, most of the candidate genes implicated in alcoholism have been related to neurotransmission (or alcohol metabolism). The model of inheritance in vulnerability to alcoholism is believed to be complex and polygenic, different subtypes of the disorder probably having different genetic aetiologies, with a varying environmental impact on the development (Bevilacqua and Goldman, 2009).

Considering the heterogeneity of alcohol use disorders, the affected study subjects in genetic studies should be classified into subtypes on the basis of available research evidence. However, only a few of the studies on candidate genes and alcoholism have been conducted this way, whereas the molecular genetic linkage studies among large family samples have yielded intermediate phenotypes. These phenotypes are more homogenous clinical subgroups of alcoholic individuals, believed to have shared neurobiology and vulnerability to the disorder. This classification of subgroups has been applied in studies to increase the power of association (Enoch et al., 2003; Schuckit et al., 2004; Goldman and Ducci, 2007; Bevilacqua and Goldman, 2009). Some of the results have also supported the existence of Cloninger's dichotomy among alcoholics, and this neurogenetic model still offers a simple way to categorize the index subjects in a clinical interview (Dick et al., 2002; Fu et al., 2002). The combined results of studies on serotonergic and dopaminergic genes in alcoholism have been equivocal. The suggested reasons for this inconsistency have included studying polymorphisms with an unknown functional impact, ethnically different study populations (even different from the controls in the same study), and studying all alcoholics as one group suffering from a homogenous disorder (Comings and Blum, 2000; Trikalinos et al., 2004).

In our studies we tried to avoid the weaknesses in the previous candidate gene studies on alcoholism. The alcoholic index subjects were classified as type 1 or 2, as Cloninger has proposed. We were studying the functional polymorphisms COMT and 5-HTTLPR, shown to affect dopamine and serotonin transmission. We also investigated whether the dopaminergic polymorphisms COMT or TaqI A, believed to affect dopamine transmission as well, had any impact on alcohol consumption among a large population sample of socially drinking males. These men, and all of the affected index subjects and controls, were white males of Finnish origin representing an ethnically homogenous group.

2 REVIEW OF THE LITERATURE

2.1 Social drinking, abuse and dependence of alcohol

What is a sensible or a "healthy" amount of alcohol to drink? Where is the limit between social drinking and abuse? Who is an alcoholic? The answers to the last two questions can be found in the diagnostic manuals. They do not list the amounts of alcohol consumed, however, only the ways alcohol has taken over the life of an affected individual. The debate about the healthy amount to drink is based on the findings of a decreased risk of cardiovascular diseases among subjects drinking small to moderate amounts of alcohol, compared with the risk of abstainers. Another reason fuelling this debate is probably the popularity of alcohol as a legal recreational substance. With respect to total mortality, the risk is illustrated as a J shaped curve, with alcohol consumed on the x axis and mortality on the y axis. When the amount consumed increases from the bottom of the J, the health hazards and total mortality significantly and rapidly increase as well. It means that drinking too much is eventually worse than drinking nothing at all. Low levels of alcohol intake (1-2 drinks per day for women and 2-4 drinks per day for men) are inversely associated with total mortality in both men and women. This was stated in a meta-analysis by Di Castelnuovo et al. (2006). Another more recent review questioned this generally accepted finding, suggesting a systematic error in prospective epidemiological studies reporting that moderate regular use of alcohol is protective against coronary heart disease. Fillmore et al. (2007) claimed that the abstainer category in most of the studies has been contaminated by former drinkers who quit because of ill health. The few studies without this error showed abstainers and moderate drinkers to be at equal risk for all-cause and coronary heart disease mortality, i.e. low levels of alcohol were not protective at all (Fillmore et al.,2007).

The limits for the safe use of alcohol, based on the available evidence, were set by Royal Colleges of Physicians, Psychiatrists and General Practitioners in Great Britain in 1995: 168 grams of ethanol weekly for a male, and 112 grams for a female, 21 units and 14 units of drink, respectively. Daily limits of not more

than three drinks for a male and two for a female have also been recommended, to avoid favouring binge drinking at weekends (Jackson and Beaglehole, 1995; Gaziano and Hennekens, 1995). The same limits have been adopted in Finland to identify excessive alcohol use. The nationally recommended limits for hazardous drinking, between social drinking and abuse, are 24 drinks weekly for a male and 16 for a female (Halme et al., 2008).

In Finland, binge drinking at weekends is a very common feature of alcohol use. The risk limits for this heavy drinking aiming at intoxication are five units for a female and seven units for a male, on one day, every weekend (Seppä, 2003). In a sense, drinking more than these recommendations is considered heavy drinking, and beyond the scale of social alcohol consumption. Inevitably there are individual differences and great variation in the risks and harms experienced by a single person in this heavy, or hazardous, drinking group; not everybody becomes an alcohol-dependent alcoholic. Still, this is a high-risk group and there is only a thin line marking the shift from heavy use to alcohol abuse. Abuse, on the other hand, has a considerable overlap with true alcohol dependence. There is no conclusive evidence of the prognosis from heavy drinking to dependence, neither are there any longitudinal studies showing the lack of that prognosis, or indicating individual protective factors (Halme et al., 2008).

The life of an alcoholic individual has been described as a cycle of 1) anticipation, 2) intoxication, and 3) withdrawal with a negative affect. The essential features of alcohol addiction/dependence are preoccupation with the next drink, loss of control over consumption, and seemingly compulsory continuation with the vicious circle despite knowledge of negative long- and short-term consequences (Koob, 2003). The aetiology of alcohol dependence is far from known, and the variation between the affected subjects is considerable, reflecting the heterogenous nature of addictive states in general. Elucidating the interactive environmental and hereditary factors that contribute to the development of alcohol dependence will permit a better comprehension of the reasons why some people are unable to control their alcohol use. It is hoped

that understanding the genetic predisposition to alcohol use disorders will enable a better understanding of the biological mechanisms involved. This in turn would provide us with a framework for the development of treatment and relapse prevention approaches. In addition, the classifications developed for genetic studies of alcoholic subjects may even help to target the treatments better than today. Of course, that would be the ideal outcome of the genetic studies as well, targeting the treatment according to the genotype of the patient (Ball and Collier, 2002).

The essential nature of substance dependence makes these disorders frustrating to treat and leads to repetitive disappointments, not just to the dependent individual and his/her near ones, but also to professionals trying to help the affected. The core symptom of dependence/addiction is relapse and the continuous risk of relapse. The risk seems to decrease during very long periods of abstinence, but apparently it never disappears. Even among medical professionals, not to mention public opinion, there is often discomfort and reluctance to apply the label of illness to substance use disorders such as alcohol abuse. They are often viewed as arising from poor self-control, character weakness, or lack of moral fibre, and are generally considered to be self-acquired states. These views are in contradiction with the scientific evidence on the nature and development of addictive states, recognizing addiction as a brain disease (Melis et al., 2005). Some of these data, the earlier dating back to the 1970's, are presented in this review below. Consistently with the disease concept, substance use disorders including alcoholism have been constantly listed and described in the international classifications of diseases for several decades. The latest versions of these classifications are reviewed next.

2.1.1 Diagnosis of alcoholism

Clinical addictions are generally diagnosed using the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R, 1987; DSM-IV, 1994) issued by the American Psychiatric Association (APA), or the International Classification of Disease (ICD) of the World Health Organization (WHO). These classifications

are generally used for research purposes, too. Both manuals recognize two categories of addictive/dependant behaviours: the less serious form of abuse, and the more serious form of dependence. Clinically, abuse is often observed to precede and predict actual dependence (American Psychiatric Association, 1987; American Psychiatric Association, 1994; WHO, 1993; Stakes, 1997).

The DSM-IV criteria for alcohol abuse are: 1) recurrent use resulting in a failure to fulfil the main obligations at work, school or home; 2) recurrent use in physically hazardous situations; 3) recurrent alcohol-related legal problems; and 4) continued use despite persistent or recurrent social or interpersonal problems caused or exacerbated by the use. At least one of the four during the previous 12 months is required, and the subject must not have fulfilled the criteria for alcohol-dependence ever. The criteria for alcohol dependence are: 1) observed tolerance to alcohol; 2) withdrawal symptoms or the need to use alcohol to avoid symptoms; 3) alcohol used in larger amounts or over longer periods than intended; 4) unsuccessful efforts to cut down the use; 5) excessive time related to obtaining alcohol or recovering from drinking; 6) impaired social, recreational or work activities because of drinking; and 7) continued use despite knowledge of the physical or psychological consequences. The diagnosis requires at least three of these seven criteria during the previous 12 months.

The former version of DSM-III-R included the same symptoms of alcohol dependence grouped differently in nine criteria, of which three were required for a diagnosis. There were fewer criteria for abuse and they were less strict than in the later DSM-IV, but essentially the same core symptoms of abuse were listed in both versions (APA 1987; APA 1994). The ICD-10 criteria for the harmful use of alcohol (synonymous with abuse) include clear evidence of physical or psychological harm which may harm interpersonal relationships. At least one month of constant use or repetitive use during the previous 12 months is required, and the symptoms must not fulfil the criteria for alcohol dependence. The criteria for dependence are: 1) an irresistible urge to drink; 2) drinking more or over a longer period than intended, or unsuccessful attempts to cut down on drinking; 3) withdrawal symptoms or use of alcohol to avoid these symptoms;

4) observed tolerance to alcohol; 5) excessive time related to obtaining and drinking alcohol or recovering after drinking; and 6) continued use despite knowledge of the physical or psychological consequences. At least three of these criteria are required during the whole of the previous month or repeatedly during the previous 12 months (WHO, 1993; Stakes, 1997).

The criteria of the DSM or ICD classification for alcohol dependence do not essentially differ, ICD-10 having been the official classification in Finland since the end of the 1990s. The diagnostic criteria used in this thesis, when appropriate, are based on the more precisely defined DSM (versions III-R and IV). Regarding genetic studies, the problem with these relatively strict classifications is that they are based on the outcome. They do not reflect aetiologies of vulnerability to alcohol abuse or dependence, but just define the endpoint of the disease process, the endpoint being a heterogeneous group of alcoholics with different aetiological backgrounds (Ducci and Goldman, 2008). The strategies to discover the genetic effects in diseases with complex aetiologies (as alcoholism has proven to be) include reclassification of the disorder into less heterogeneous subtypes, such as using Cloninger's typology or defining intermediate phenotypes. These methods are discussed below in the relevant sections. In this dissertation "alcohol abuse" and "alcohol dependence" are included in "alcohol use disorders". Abuse and dependence are commonly used as synonyms in clinical contexts and records, but dependence actually is a more advanced and a more precisely defined state of abuse. In a diagnostic context there is a clinical overlap even when using DSM criteria. "Substance use disorders" here includes all substances of abuse, also alcohol. "Alcohol dependence" and "alcoholism" and "alcohol addiction" are used as synonyms. An "alcoholic" is an "alcohol dependent" individual.

The Michigan Alcoholism Screening Test (MAST) was originally developed and validated as a structured interview instrument to rapidly detect alcoholism with 25 simple "yes" or "no" questions. It has been reliably used as a self-administered aid for a clinical interview, as was done to screen the type 1 alcoholic subjects in this study. Even the validation study found the number of

false positives to be very low; MAST does not often identify a non-alcoholic as an alcoholic (Selzer, 1971). Alcohol consumption of the socially drinking subjects in these studies was assessed with a structured quantity and frequency method from the Nordic alcohol consumption inventory and its modification (Hauge and Irgens-Jensen, 1981; Kauhanen et al., 1992).

2.1.2 Diagnosis of antisocial personality disorder

To classify the alcoholic subjects in this study (type 1 versus type 2 alcoholism), they were screened for antisocial personality disorder (ASPD) according to the DSM-IV criteria, as well as for alcohol dependence. The criteria for antisocial personality disorder describe a pervasive pattern of disregard for and violation of the rights of others occurring since the age of 15 years: 1) failure to conform to social norms with respect to lawful behaviours (arrested repeatedly); 2) deceitfulness (indicated by repeated lying, or conning others); 3) impulsivity or failure to plan ahead; 4) irritability and aggressiveness (repeated physical fights or assaults); 5) reckless disregard for the safety of self or others; 6) consistent irresponsibility (failure to sustain consistent work behaviour or fulfil financial obligations); and 7) lack of remorse (indifferent or rationalizing behaviour after having mistreated or stolen from others). At least three of these criteria must be present. The individual has to be at least 18 years of age when diagnosed, and there has to be evidence of conduct disorder before the age of 15. Conduct disorder is defined as a repetitive and persistent pattern of behaviour in which either the basic rights of others or major age-appropriate social norms or rules are violated (including different forms of aggression to people or animals, destruction of property, deceitfulness or theft, and serious violations of rules) (American Psychiatric Association, 1994).

Given the similar pattern of symptoms, it is not difficult to see that conduct disorder in a subject less than 15 years of age predicts antisocial personality disorder in the adult. The antisocial personality disorder shares many of its traits with Cloninger's type 2 alcoholism described in a section below.

2.1.3 Epidemiology and co-morbidity of alcoholism

The lifetime prevalence of alcohol dependence or abuse in the USA in 1990 was estimated to be 13.5% in a large epidemiological catchment area (ECA) study, in which over 20 000 persons were interviewed. Almost 40% of those suffering from alcohol disorder also suffered from another mental disorder, the highest rates of specific disorders being affective, anxiety or antisocial personality disorder. Among those with a mental disorder, the lifetime prevalence of alcohol disorder was 22% (Regier et al., 1990). In another study among 928 male alcoholic patients in treatment, over 60% of the subjects fulfilled lifetime criteria for at least one other additional mental disorder. Affective and anxiety disorders as well as antisocial personality and drug abuse were the most frequently identified (Penick et al., 1994).

Kessler et al. (1994) published another ECA study in the National Co-morbidity Survey (USA), where over 8000 participants were interviewed. Nearly 50% reported at least one lifetime DSM-III-R disorder, the most common being major depressive disorder, alcohol dependence, and social or simple phobias. The lifetime prevalence of alcohol dependence for both sexes was 14% and that of abuse almost 10% (together over 23%). More than half of all the lifetime disorders were concentrated in one-sixth (14%) of the study population with three or more co-morbid disorders. The lifetime prevalence of antisocial personality disorder among males was 5.8% (Kessler et al., 1994). (The co-morbidity of antisocial personality disorder with alcohol dependence has a crucial impact in the development of type 2 alcoholism, as discussed below in the section on Cloninger's neurogenetic model). The same group of researchers replicated the survey in 2001–2003 in the same area in Michigan, USA, interviewing over 9000 participants. The most prevalent lifetime disorders again were major depressive disorder (16.6%) and alcohol abuse. Almost one-fifth of the study population (18.6%) suffered from alcohol abuse (13.2%) or dependence (5.4%), and about 50% had at least one lifetime disorder (Kessler et al., 2005). The estimates of the prevalence of substance abuse, including alcohol, were consistent with those reported elsewhere in the world in 2000–

2004 (Demyttenaere et al., 2004). The estimates for alcohol disorders were lower in this later survey than in the earlier one (just under 20% versus a little over 20%). The authors speculated that this was due to the more strict criteria for substance dependence in the DSM-IV classification used in the later study (Kessler et al., 2005).

In 2004, the World Health Organization (WHO) estimated that 2 billion people consume alcohol, 1.3 billion use tobacco, and 185 million use illicit drugs worldwide. The number of subjects with alcohol use disorder was estimated to be almost 80 million (Bevilacqua and Goldman, 2009), It has been estimated on a population basis that alcohol alone subtracts an average of 4 disability-adjusted life years per person (the years of life lost due to premature death or disability), the same as tobacco, whereas illicit drugs subtract less than one year. For comparison, insulin-dependent (type 1) diabetes subtracts only 0.1 years. The addiction disease burden has a higher impact in the industrialized Western world than in the developing countries, where life expectancies are shorter (Merikangas and Risch, 2003).

2.1.4 Alcohol consumption, alcohol disorders and public health in Finland

In 2006, 88% of the adult population in Finland reported using alcohol. Of these, the number of heavy consumers is estimated to be even 500 000 (6–12% of the adult population). This 10% of drinkers with the highest consumption account for almost half of the alcohol (over 40%) consumed in Finland. The percentage of alcohol consumers is greatest among young adults and middle-aged people. About 10% of adults of both sexes are abstainers. Men tend to use alcohol more often and to consume more drinks on one occasion than women. Most young people start to experiment with drinking between the ages of 12 and 16 years, with only one-fifth of 16-year-olds not using alcohol at all (Nylander et al., 2007).

In Finland, alcohol consumption was well below the mean in other European countries up to the 1960s. After new alcohol legislation in 1969, allowing among other things the sale of medium strong beer (class III) in grocery stores,

consumption in Finland more than doubled in ten years. Thereafter there was a more or less steady increase during the upswing in the economy in the1980s, and this has continued since the end of 1990s after a temporary decrease. The latest rapid increase in consumption and adverse effects of the increase were seen after the lowering of taxes on alcoholic beverages at the beginning of 2004. Connected to this, the quotas on tax-free imports of alcohol from other EU countries to Finland were abolished, and nearby Estonia with very low retail prices on alcohol joined the EU, enabling cheap and rather unrestricted importing of alcohol. In 2006 the real retail prices (adjusted for inflation) in Finland were still 20% lower than in 2003 (Nylander et al., 2007).

In 2006 the total alcohol consumption of an average adult was 10.6 litres of 100% alcohol. Again, the total consumption has more than doubled over the past three decades, since the first boom in the 1970s. This has brought Finland to the medium level of alcohol consumption among other European countries. Compared with them, binge drinking (heavy intoxication) continues to be more common in Finland. One man in four reported drinking at least six units of alcohol on one occasion weekly, and one woman in eight at least four units on one occasion weekly. One man in six, but only one woman in twenty, reported being heavily intoxicated at least once a month during the previous year (Mäkelä, 2003; Nylander et al., 2007). The prevalence of hazardous, heavy drinking possibly preceding abuse or dependence at least in some cases was 5.8% in a Finnish study, being more prevalent among middle-aged, divorced, and unemployed males (Halme et al., 2008). The authors thought that this very probably is an underestimation.

In an annual postal survey follow-up carried out by the National Public Health Institute (KTL) between 1978 and 2006 among working-age citizens in Finland, the trends in chronic disease-related health behaviours were mainly positive concerning smoking, food habits and leisure time activities (Helakorpi et al., 2007). However, overweight had become more prevalent, and the consumption of alcohol had steadily increased. The difference in consumption between educational groups had disappeared among men, but highly-educated women

were drinking slightly more than those with a lower educational level (Helakorpi et al., 2007).

Mortality rates among heavy drinkers and alcoholics are claimed to have increased at least two-fold, or even up to eight-fold compared with the general population. On the other hand, the number of deaths related to alcohol has generally been underestimated in the statistics (Saarnio and Mäkelä, 1997). The number of alcohol-related deaths was estimated to be 3050 in 2006, and there has been an increase of over 20% in the seven years since 1999, and an especially rapid growth since 2004. The increase was exclusively due to alcohol-related diseases or alcohol poisonings, since the number of fatal accidents, or violence with alcohol involved, had not changed. Alcohol-related causes were the most common cause of death among working-age (15–64 years) males and females in Finland in 2006. In addition, using a broader definition alcohol was a contributory cause of death in 1600 more cases (Nylander et al., 2007).

Inpatient wards registered over 36 000 periods of care with an alcohol-related disease as a primary or secondary diagnosis in 2006. Traditionally, a strong link has existed between alcohol use and violent offences in Finland. In recent years, 70–80% of the fatal violent offences and assaults have been committed under the influence of alcohol. In 2006 over 31 000 violent offices were recorded by the police in Finland. Almost 26 000 cases of drunk driving were detected by the police, and alcohol was involved in one in four fatal road traffic accidents (Nylander et al., 2007).

It was estimated that in 2005 Finnish society paid almost one million euros in direct costs, and between three and six billion in indirect costs as a result of alcohol use. A third of the direct costs were caused by public disturbances. Health care accounted for a quarter, and social services for a one-fifth of the direct costs (Nylander et al., 2007). Reducing the level of alcohol consumption and preventing related harms has been the main objective of Finnish alcohol policy also in recent years. This seems reasonable considering the costs and the diverse negative effects of alcohol on the health of the Finnish population,

specifically of the heaviest drinkers. Still, less than half of the municipalities have an alcohol and drug strategy of detoxification or other treatments. Only half of the municipalities in Finland can offer immediate access to a detoxification program, and in one in ten municipalities there is no available detoxification treatment at all (Nylander et al., 2007).

2.1.5 Treatment of alcoholism

The clinical outcome of alcoholism after a standard detoxification treatment is known to be poor, even if psychosocial therapy is implemented, with up to 70% of patients resuming drinking within one year (Johnson, 2008). Based on 30 years of research, it can be concluded that individuals obtaining treatment for their drinking problem have a slightly better outcome than those who are not. However, the results of follow-up studies over a period longer than one year suggest very low long-term effectiveness of the treatments available. There seems to be a little if any difference in the effect between the types of therapies offered, longer and shorter duration of treatment, or between medical in-patient and non-medical out-patient treatment. In addition, several large studies have shown the traditional Twelve Step Facilitation of Alcoholics Anonymous to still be as effective as the more modern and costly cognitive and behavioural therapies (Room et al., 2005).

There has been growing interest, even occasional enthusiasm, in developing pharmacological therapies to improve the treatment effects of alcohol dependence, as an adjunct to psychosocial therapies. The medications tried so far have shown only a modest additive effect, however, and the efficacy usually is seen only in limited subpopulations of the treated alcoholics. The tested medicines include disulfiram, naltrexone and its depot formulations, acamprosate, topiramate, SSRIs (selective serotonin re-uptake inhibitors), ondansetron, and quetiapine. As an example, acamprosate is considered to hold a substantial value for an extra treatment effect with a 13% overall improvement in twelve months abstinence rates. (This would generally be judged as modest pharmacological efficacy of treatment.) Molecular genetic

studies are expected to help in targeting the existing pharmacological treatments at different types of alcoholics for better response, and to develop completely new treatments by detecting the differences in the neurobiology of the various forms of alcoholism (Goldman et al., 2005b; Room et al., 2005; Johnson, 2008).

2.2 Cloninger's neurogenetic model of alcoholism (types 1 and 2), antisocial personality disorder (ASP) and impulsive violent behaviour

One of the pioneers of alcohol research, Jellinek (1960), distinguished different subgroups of alcoholism, such as the individuals who had an "inability to abstain entirely", or those who could abstain even for longer periods but were unable to stop drinking binges once they started, and thus suffered from "loss of control". In spite of this, many clinical and developmental studies of alcoholism generally, even until recently, have used heterogenous samples of subjects without subgroups. This has made the findings difficult to interpret and replicate. On the other hand, alcohol abuse has a strong familial aggregation, alcoholism being three to five times as frequent in parents, siblings and children of alcoholics as in the general population. Based on this and further studies of Swedish adoptees and families in the United States, Cloninger proposed the dichotomy of alcoholism in two subtypes based on their clinical features and patterns of inheritance (Cloninger, 1987; Cloninger, 1995).

The two subgroups (type 1 and 2) can be distinguished in terms of a tri-dimensional combination of heritable personality traits: novelty seeking, harm avoidance and reward dependence. Cloninger noted that the subtypes should not be considered as discrete disease entities, and an individual alcoholic may have features of both types. There are polar extremities of both subtypes among alcoholics, but generally the features of the three personality traits form a continuum. Type 1 alcoholics most typically are high in harm avoidance and reward dependence, but low in novelty seeking. True type 2 subjects exhibit the opposite traits: high in novelty seeking, but low in harm avoidance or reward dependence. The findings of the adoptee studies indicated that the risk for type 1

alcoholism was increased only if there was both a genetic (inherited) predisposition and a familial, environmental exposure to heavy drinking. Because it requires both kinds of predisposition before the risk is increased (more than doubled in both sexes of the adoptees), type 1 alcoholism has been described as "milieu-limited". In contrast, the adopted-away sons of fathers with early-onset spontaneous alcohol-seeking behaviour (type 2 alcoholic fathers) did not need environmental provocation to develop similar addictive behaviour early in life. The risk for alcoholism in these sons was nine-fold that in the sons of other fathers. Because the daughters of type 2 fathers were at higher risk only for somatic anxiety but not for alcoholism, type 2 alcoholism has been called "male-limited" ("from father to son") (Cloninger, 1987; Cloninger, 1995). It has been estimated that 80% of alcoholics in general fall into the type 1 category (both males and females) and the remaining 20% into the type 2 category (almost exclusively only males) (Tupala and Tiihonen, 2004).

The two types were further characterized by opposite definitions and descriptions: type 1 has an adult onset after 25 years of age, but a rapid development of dependence on the anti-anxiety effects of alcohol. This leads to alternating drinking binges (loss of control and guilt feelings) and periods of abstinence, without prominent antisocial or impulsive violent behaviour. Type 2 has a teenage onset, at the latest before 25 years of age, no guilt feelings for the drinking or no desire to quit. Type 2 alcoholics also have a propensity to abuse different kinds of drugs for their euphoriant effects, not just alcohol. They are also prone to recurrent crime and impulsive violent outbursts from a young age, especially under the influence of alcohol. Type 2 males are risk-taking adventurers seeking for occasional euphoria in a selfish manner, optimistic but antisocial and even vengeful in nature. They are also described as aloof, lacking compassion and empathy, and non-sensitive to reward or punishment starting from childhood (conduct disorder). At the opposite pole, type 1 alcoholics are anxiety-prone, deliberate, cautious, reward-dependent and friendly. They are socially dependent and empathic, but also worried, fearful and pessimistic (Cloninger, 1987; Cloninger, 1995). The two types are often

described and compared by a pair of words: a worrier and a warrior, type 1 vs type 2 (Goldman et al., 2005a).

In a study of 171 primary alcoholic males, Irwin et al. (1990) confirmed the clinical importance of age at onset of symptoms of alcohol abuse. In their study, younger age at onset was significantly associated with more severe alcohol-related life problems later on, abuse of other drugs and childhood criminality. These same high-risk subjects also more often met the criteria for adult ASP, though subjects with a primary diagnosis of ASP were originally excluded from the analysis. Thereafter, the comparison between Cloninger's subtypes 1 and 2 did not reach significance. The authors concluded that differentiation between type 2 alcoholism and ASP may not be meaningful, but these two disorders may share the same aetiology: type 2 alcoholics actually suffer from ASP, while alcohol-related problems such as impulsive violence are only one part of the syndrome (Irwin et al., 1990). Cadoret et al. (1995) found evidence of two genetic pathways to drug abuse/dependency in their study among 95 male adoptees. One pathway went directly from a biological parent's alcoholism to the adoptee's drug abuse/dependency. The second path was circuitous, starting from the antisocial personality disorder in the biological parent, proceeding through adoptee aggressivity, conduct disorder, antisocial personality disorder, and eventually ending up in drug abuse or dependency. The results suggested the existence of two inheritable forms of alcoholism, resembling Cloninger's types 1 and 2 (Cadoret et al., 1995).

In the National Longitudinal Alcohol Epidemiologic Survey in 1992 in the USA, almost 43 000 participants were interviewed for drug and alcohol use disorders. The study found the age at first drug use/drink to be a powerful predictor of lifetime abuse problems among both sexes: the odds of lifetime abuse or dependence among the sample of lifetime users was reduced 4–5% for each additional year that drug use onset was delayed. The reason for this was not clear. Either there was a combination of risk factors mediating the protective effect of the delay, or the early start was an indicator of the inevitable development difficult to modify (Grant and Dawson, 1998). Other researchers

have also suggested that the early start of alcohol use is not necessarily the reason for developmental problems in a child and it might be just one of the manifestations of general maladaptive behaviour (Prescott and Kendler, 1999).

Cloninger originally proposed in his typology that a dopaminergic deficit would be related to type 1 and serotonergic deficit to type 2 alcoholism (Cloninger, 1987; Cloninger, 1995). Offenders with ASP are found to have low mean concentrations of 5-hydroxyindolacetic acid (5-HIAA, the main metabolite of serotonin) in their cerebrospinal fluid (CSF), indicating lower than normal serotonin turnover in the brain (Linnoila et al., 1983; Virkkunen et al., 1989; Virkkunen et al.,1996a). The primary behavioral traits correlating with low CSF 5-HIAA have been found to be increased irritability, impaired impulse control and stimulus (novelty) seeking, along with disturbed diurnal activity rhythms (Virkkunen et al., 1996b). Possibly, as a compensatory mechanism to the low brain serotonin turnover (the low CSF 5-HIAA), entry of the amino acid tryptophan (precursor of serotonin) into the brain increases. This may result from the increased plasma insulin levels (Virkkunen and Narvanen, 1987; Tiihonen et al., 2001; Virkkunen et al., 2009).

Brain imaging studies also implicate deficits in serotonergic neurotransmission in type 2 alcoholic subjects (Tiihonen et al., 1997). On the other hand, brain imaging studies have found a dysfunction in dopaminergic neurotransmission among type 1 alcoholics, mostly indicating decreased transmission (Hietala et al., 1994; Volkow et al., 1996; Tiihonen et al., 1998). Tiihonen et al. (1995) found alterations in striatal dopaminergic function (transporter densities) among both types of alcoholics but in opposite directions: markedly lower dopamine turnover in type 1, and slightly higher in type 2, compared with healthy controls.

Impulsivity is generally considered deleterious to normative, adaptive functioning and social interactions. Gerald and Higley (2002) studied nonhuman primates (rhesus monkeys) and speculated that the benefits derived from impulsivity may have maintained the genotypes and phenotypic expression of low 5-HT turnover. In primate societies low 5-HT activity has been linked to

impulsive, risk-taking and dangerous behaviour, including high rates of alcohol consumption if available. The researchers observed that males with low CSF 5-HIAA leave their natal group at a younger age to seek sexual opportunities. The more inhibited primate males staying in the group are less likely to reproduce ("nothing ventured, nothing gained"). The quick-tempered aggressiveness may also serve as a life-saving behavioural trait for a solitary male, and also as an aid in fighting over females. In primate societies the males with low brain 5-HT levels reproduce younger than their mates with higher levels of CSF 5-HIAA. They may "live fast, die young". (This is a clinical picture observed in antisocial, impulsive aggressive human males as well.) Natural selection can maintain such genotypes leading to earlier fertility at the expense of later survival, because the individual may have already reproduced at the time when the genes exert their damage (premature death) (Gerald and Higley, 2002).

Since Cloninger's first published version of his typology, several researchers have formulated new ones with two to four subclasses of alcoholism, based on different theoretical backgrounds. Those with two subtypes have a close resemblance to Cloninger's dichotomy, even though identified in different samples and within different ethnic groups (Babor et al., 1992; Schuckit et al., 1995). Those including more than two categories (most often a chronic/severe type, a depressed/anxious type, a mildly affective type and an antisocial type) also try to cover the alcoholic individuals who would not fit in the strict polar extremities of a pure dichotomy. Unfortunately few of the typologies have been examined longitudinally to test their clinical value and predictive utility (Hesselbrock and Hesselbrock, 2006). However, Babor's classification of type A and B alcoholism, as a broader concept of alcoholism than Cloninger's dichotomy, is a common tool in alcohol research these days (Roache et al., 2008).

Cloninger's dichotomic classification was supported by the Collaborative Study on the Genetics of Alcoholism (GOGA), which found a significant replicated linkage to chromosome 1 for a quantitative phenotype related to aspects of alcohol use (resembling that of Cloninger's type 1 subjects) and

anxiety. In the same study, two of the symptom clusters (factors) identified and related to the diagnosis of alcohol dependence corresponded to Cloninger's type 1 and type 2 alcoholism, accounting for 14% and 41% of the variance, respectively (Dick et al., 2002). In a twin study by Fu et al. (2002) using the Vietnam Era Twin Registry, 3360 military veteran male twin pairs (of which almost 2000 were monozygotic and 1500 dizygotic) were interviewed to screen for lifetime DSM-III-R diagnosis of antisocial personality disorder (ASPD), alcohol dependence (AD), marijuana dependence (MJD), and major depression (MD). The results indicated that a substantial proportion of the genetic variance in the risk of AD and of the total variance (genetic and shared environmental) in the risk of MJD were accounted for by the genetic effects associated with ASPD, which appeared to be the major determinant of risk of substance dependence. The researchers suggested that the temporal ordering of the disorders started from ASPD, MD and substance dependence (AD, MJD) being secondary to ASPD. There was a strong comorbidity between ASPD and MD as well (Fu et al., 2002).

2.3 Neurobiology of alcoholism

The effects of alcohol in the brain are considered non-specific, and in animal studies acute alcohol administration has been found to increase the action of the neurotransmitters GABA (gamma-aminobutyric acid), glutamate, dopamine, serotonin and opioid peptides. Of these, dopamine has been most strongly associated with the reward pathway, and is considered crucial for the reinforcing effects of alcohol, leading to repetitive recreational use, abuse or sometimes compulsory use of alcohol, called dependence or alcoholism. Stress-associated hormonal changes also may contribute to the development and maintenance of dependence, especially the hypothalamus-pituitary-adrenal cortex axis (HPA axis), and corticotropin releasing factor (CRF) and its binding protein (CRH-BP) (Enoch et al., 2008a). The involvement of multiple neurotransmitters, not just dopamine, in reward mechanisms is very probable. The actions and co-actions of different transmitters have been hypothetically

described as a reward cascade, where the release of dopamine still is considered a crucial point (Koob and LeMoal, 2001; Bowirrat and Oscar-Berman, 2005). Regarding the effects of alcohol, the brain dopamine, serotonin and glutamate turnover and function have been the object of extensive research. They are discussed in the next sections, and the functions of other transmitters listed above more briefly in the relevant sections related to candidate gene association studies below.

2.3.1 Brain dopamine function, reward mechanisms and alcoholism

The crucial role of the catecholamine neurotransmitter dopamine (DA) in the brain reward mechanisms is well established. Natural reward feedback is essential for the survival of the individual and the species. The individual tends to return and repeat the rewarding and reinforcing action, even with some trouble, once having learned it. Natural sources of reward include food, sexual interaction, positive social interactions and brain stimulation by humour or other pleasure. Dopamine also mediates the rewarding feelings from unnatural sources such as addictive drugs including heroin and other opiates, cocaine, amphetamine, nicotine and alcohol. Dopamine has been called the brain's "pleasure chemical". However, dopamine may not be the only transmitter mediating reward, though dopaminergic pathways are often referred to as the "brain's reward circuit" (Koob, 2003; Melis et al., 2005).

DA is synthesized in the neuron and once released into the extraneuronal synaptic cleft it binds to the post- and presynaptic receptors, evoking biological events which can result in the rewarding psychological experiences described above. DA receptors are divided into two subtypes, or families: D1-like (D1 and D5) and D2-like (D2, D3, and D4). The D2-like, predominantly D2, is found at high levels in typical DA-rich brain areas, such as the striatum and nucleus accumbens (NAC). In cortical regions, D1 receptors are the more prominent receptor subtype. DA reuptake carrier or dopamine transporter (DAT) is a protein that terminates the action of released DA and regulates its concentration by collecting and eliminating DA from the synaptic cleft. In the presynaptic

neuron, DA is inactivated by the enzyme catechol-O-methyltransferase (COMT, see the relevant section below) (Tupala and Tiihonen, 2004).

Drugs abused by humans stimulate dopamine release from the NAC in the brain's mesolimbic system, and this is postulated to be the main neural network mediating the reinforcing effects of drugs and alcohol. The NAC is speculated to act as a filter, or gate, between the brain regions controlling moods or drives and action (Tupala and Tiihonen, 2004). Numerous animal studies have shown ethanol (alcohol) to cause a dose-dependent release of DA in the rat brain NAC, and withdrawal from ethanol decreases the release. The animals will reinstate the rewarding DA release on the prewithdrawal level by self-administering alcohol, if possible. It has also been demonstrated that just the anticipation or the expectancy of alcohol elicits reward-related DA activity in the NAC of the rat. This possibly is an indication of a more or less permanent change caused by neurochemical reinforcement after repeated alcohol administration (Koob and LeMoal, 2001). In human studies the subjective perception of pleasure induced by the cocaine-like psychostimulant drug methylphenidate has correlated positively with the levels of DA released, measured with positron emission tomography (PET) using radioligand (Volkow et al., 1999).

The animal studies after experimental brain lesions seem to suggest that lower levels of DA activity are associated with higher levels of consumed alcohol, possibly to restore the artificial dopaminergic deficit - the disturbed "reward balance". The studies with inbred ethanol-preferring and non-preferring rat strains mostly indicate that the density of D2 receptors in limbic areas might be lower in ethanol-preferring animals (Tupala and Tiihonen, 2004). (The preferring strains act as a model of alcoholism in humans.) Phillips et al. (1998) showed a markedly decreased alcohol preference in mice knock-outs totally lacking functional D2 receptors, which also underlined the importance of intact DA signalling and D2 receptors concerning ethanol-related behaviours.

In human studies the growth hormone response to the DA agonists apomorphine or bromocriptine has been lower in alcoholic subjects, supporting

the theory of an association between decreased DA transmission and alcoholism. DAT binding is considered to be a marker of DA neuron terminals, and the whole hemisphere autoradiography (WHA) studies have revealed lower DAT densities among type 1 alcoholics. Also, the density of D2 receptors in WHA studies seems to be lower among type 1 alcoholics. These findings might explain why some humans (the type 1 alcoholics), because they experience natural reinforcing and rewarding mechanisms as insufficient, are more vulnerable to more frequent consumption and higher doses of alcohol than the non-addicted general population (Tupala and Tiihonen, 2004).

2.3.2 Brain serotonin (5-HT) function, alcoholism and violent behaviour

Monoamine neurotransmitter serotonin and its receptors are found both in the central nervous system and peripherally in the human body. Within the brain, the raphe nuclei contain the cell bodies of 5-HT neurons with projections to various regions in the brain. 5-HT is synthesized in the neuron from amino acid tryptophan, the enzyme tryptophan hydroxylase catalyzing the rate-limiting step in the conversion. The gene coding for the tryptophan hydroxylase responsible for this reaction in the human brain is called TPH2 (Walther and Bader, 2003). Once released extracellularly from the presynaptic vesicles, 5-HT binds to postsynaptic receptors transmitting a signal. 5-HT also binds to presynaptic 5-HT1A and 5-HT1B/D autoreceptors, which modulate the further release of 5-HT. The serotonin transporter protein (5-HTT) is responsible for the reuptake of 5-HT out of the synapse and for the termination of the synaptic transmitter action of 5-HT. In the presynaptic neuron, monoamine oxidase-A (MAO-A) inactivates intracellular 5-HT, thus regulating its levels. The 5-HT receptor family consists of 7 known subtypes of 5-HT1-7, some with subtypes of their own, such as 5-HT1A-F (Veenstra-VanderWeele et al., 2000).

In animal studies including alcohol-preferring or non-selected rat strains, a sizeable number of studies support the notion that facilitating serotonergic transmission decreases ethanol intake. Also, the blockade of 5-HT-3 or 5-HT-2 receptors with antagonists results in decrease of intake. It remains unclear

whether decreasing transmission would actually increase ethanol intake in animals. Acute ethanol intake most probably transiently increases the brain's 5-HT levels, and possibly transmission as well. As a consequence, this would activate the mesolimbic dopaminergic reward system, too (LeMarquand et al., 1994). The acute effect of ethanol might be biphasic, though, with the increase followed by a decrease in 5-HT transmission leading to behavioural disinhibiton in animals. In a case of chronic intake, the serotonergic system more likely adapts with successive doses of alcohol, and the net effect in transmission remains minimal or negative (LeMarquand et al., 1994). In contrast, after withdrawal of chronic ethanol administration, a decrease in brain serotonin transmission occurs. In animals, this increases the sensitivity of the neural system to perturbation by exogenous stimuli, disturbing the ability to maintain self-organization, and leading to impulsive non-inhibited behavioural reactions (LeMarquand et al., 1994). An almost consistent finding, however, is that the levels of CSF serotonin and dopamine metabolites are positively correlated with one another, and serotonergic stimulation facilitates dopamine release, at least when basal activity is low (Jibson et al., 1990).

The serotonergic projections from raphe nuclei to the forebrain are considered crucial in human behavioural inhibition and impulse control. Serotonin often modulates dopaminergic activity, too. The hypothalamic area with projections from the suprachiasmatic nucleus is supposed to control individual carbohydrate intake, and the suprachiasmatic nucleus is thought to act as the major circadian pacemaker ("the intrinsic clockwork"). This nucleus receives a serotonergic input from the raphe nulei in the brain stem, so there might be an anatomical and serotonergic link between the regulation of the sleep-wake cycle, regulation of blood glucose metabolism and impulse control. Disruptions of these functions, along with disturbances in brain serotonin functions, are common among persons with antisocial personality disorder (ASP) and habitual impulsive violent behaviour. The same subjects most often also suffer from alcoholism, referred to as type 2 (Virkkunen et al., 1994a; Virkkunen et al., 1994b).

2.3.3 Brain glutamate (Glu) function, NMDA receptors, and alcoholism

Approximately half of the synapses in the brain are excitatory synapses that use glutamate as their neurotransmitter. The glutamergic neurons are most abundant in the cerebral cortex and limbic regions. The postsynaptic glutamate receptors are divided into ionotropic receptors, including NMDA (N-methyl-D-aspartate), AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate, and into metabotropic receptors. Synaptic concentration of glutamate is partly regulated by glutamate aspartate transporter (GLAST). Glutamate activity at NMDA receptors is believed to mediate associative learning (Enoch, 2003; Schumann et al., 2008).

Glutamate receptors are primary targets of alcohol action, and acute intoxication inhibits the NMDA receptor complex, by reducing the effect of glutamate and thus impairing learning and memory. This impairment is evident even at the low blood alcohol levels associated with social drinking. This also logically explains blackouts associated with acute intoxication. Reduced glutamate activity in the hippocampus area leads to temporal impairment of spatial memory. On the other hand, there have been observations of acute low doses increasing extracellular glutamate temporarily. This makes the effects of alcohol on glutamate transmission paradoxical and ambiguous (Gass and Olive, 2008). Chronic abuse of alcohol causes sensitisation (upregulation) of NMDA receptor subunits, mainly the units NR1, NR2 and NR2B. These changes in neurotransmission may contribute to the hyperexcitatory symptoms observed during withdrawal (e.g. seizures), and hyperexcitability after chronic use may contribute to craving and relapse behaviour as well. Consequently, the effects of alcohol on glutamate NMDA receptors are associated with every phase of the alcohol disorder: intoxication, withdrawal, tolerance, dependence, craving, and relapse (Gass and Olive, 2008).

2.3.4 Long-term effects of alcohol on neurotransmission and reward thresholds: tolerance, dependence, withdrawal, craving and relapse

A considerable amount of data has been gathered on the acute reinforcing effects of alcohol. The data are not conclusive, but even less is known about the long-term mechanisms involved in the development of dependence and tolerance. In particular, there has been a lack of data regarding relapse after periods of variable length of abstinence. Moreover, the choices of medical treatments for effective relapse prevention are few. The long-term effects of alcohol ingestion on neurotransmission are probably different from the acute effects. In animal studies the changes during long-term intocixation have been even reverse to ones observed during acute ingestion. This would actually mean a decrease in the function of neurotransmitters such as dopamine, serotonin, GABA and glutamate, associated with chronic use of alcohol. This leads to an elevation in brain reward thresholds, a possible explanation for tolerance and for the need to increase the dose observed in dependent states. The animals try to compensate for this shift in reward balance by self-administering ethanol, if they are allowed to do so (Koob and LeMoal, 2001; Koob, 2003).

Koob and LeMoal (2001) have proposed a model for the brain changes occurring during the development of addiction that explains the persistent vulnerability to relapse long after the compulsory abuse has ceased. At the beginning of the abuse, drug seeking is driven by positive reinforcement of the actual use and the anticipation of use, resembling a classical impulse control disorder. When the drug taking (or drinking) continues, the negative affects become more prominent over time. The addiction becomes driven by this negative inforcement instead of the positive one felt at the beginning. The addiction also takes on characteristics of a compulsive disorder, presumably recruiting the same neural circuits associated with such a disorder (cortico-striatal-thalamic loop). An addicted person, like one suffering from compulsive disorder, performs repetitive behaviours to reduce anxiety and distress, not to provide pleasure or to gain a reward. This leads to activation of the brain stress

system while the reward system is in a constant under-activated state (Koob and LeMoal, 2001).

One of the direct mediators of stress is corticotrophin-releasing factor (CRF). Increased levels of circulating glucocorticoids apparently have a minor impact. The shift from normal homeostasis to a less rewarding, more stressful state was termed a shift to allostasis by Koob and LeMoal (2001): achieving stability through change, but leading to chronic deviation of the normal reward thresholds. According to the hypothesis, the reward or stress systems never return to the original states, even if some normalisation occurs during protracted abstinence periods. The activated brain stress system mediated by CRF and the negative affective state observed in dependence and during acute withdrawal remain as more or less permanent changes. Consequently, once-addicted subjects are constantly more vulnerable to relapse than they were before they started the abuse in the first place. This is reflected as a symptom of craving even after long periods of abstinence (Koob and LeMoal, 2001). In animal studies it has been shown that even the environmental stimuli predicting reward and not just the reward itself (e.g. the taste of alcohol) can activate mesolimbic dopaminergic transmission. Indications of similar changes have been found in the brain imaging studies of alcoholic human subjects, too (Weiss and Porrino, 2002). These findings of course have direct implications for the increased risk of relapse in different contexts and environments.

2.4 Genetics of alcoholism

The individual vulnerability or resilience to alcohol use disorders can be both genetic and environmental, such as availability of alcohol, positive parental or peer support or lack of it. Genetic vulnerability factors for alcoholism have been divided into three categories: 1) heritable personality traits may predispose the individual to seek out and consume alcohol, increasing the chances of becoming addicted (traits such as anxiety or impulsivity); 2) an inherited differential response to alcohol affects one's vulnerability, e.g. low response to the sedating effects of alcohol facilitates drinking large quantities and increases

the risk for alcoholism; 3) genetic variation in neurobiological pathways (transmission) may render some individuals more vulnerable to the loss of control, such as defects in the natural reward mechanisms of the brain. For each individual the balance between environmental and genetic risk factors and their constant interplay is different and even varies between different phases of life (Enoch, 2003).

2.4.1 Family, twin, and adoption studies and transmission of addiction

Alcohol dependence has been traditionally known to cluster in families. Plutarch (as early as AD 45–125) stated "ebrii gignunt ebrios" ("drunks beget drunkards"). Much later it was shown in a more empirical fashion that among 6250 alcoholics and 4100 non-alcoholics, an alcoholic was six times more likely to report parental alcoholism (Cotton, 1979) or that the rates of alcoholism were doubled in relatives of alcoholics compared with that in controls (Guze et al., 1986). Dawson et al. (1992) calculated the risks from a study of over 23 000 drinkers: the increase was 170% for an individual with both a first- and second-degree alcoholic relative, almost 100% with a only first-degree alcoholic relative, and still almost 50% for an individual with only second or third degree relative affected. However, family studies do not distinguish between genetic and shared environmental effects (Goldman et al., 2005a).

The basic presumption behind twin studies is that monozygotic twins, sharing all of their genes in common, are more similar in phenotype than dizygotic twins, who on average have only 50% of their genes in common. The studies among di- and monozygotic twins between 1960 and 1997 gave typically figures of 0.5 for males and 0.25 for females as an estimate of heritability in alcoholism. Goldman et al. (2005a) also calculated the heritability as 0.5, derived from a data of almost 10 000 twin pairs. This would mean that the genetic and environmental factors are equally important in determining the risk for alcoholism (50–50%) (Ball and Collier, 2002; Goldman et al., 2005a).

Adoption studies provide a different view of the interaction between environmental and genetic factors, looking at individuals reared by unrelated

parents. (Some aspects of this were discussed above in the section on Cloninger's neurogenetic model of alcoholism.) Adoption studies in 1945–1987 provided strong evidence for the action of genetic factors, especially among males. There seemed to be no correlation between the drinking behaviour in the adoptees and alcoholism in the foster family, nor any protective effect of being raised away from the biological parent (Ball and Collier, 2002). It has also been suggested that women with the same degree of genetic risk are less likely than males to become alcoholics. Perhaps there has to be more interplay between genes and environment for women to reach a risk threshold to develop alcoholism (Quickfall and el-Guebaly, 2006). On the other hand, it has been claimed that alcoholism is just as heritable in women as in men, and that the detection of genetic influences in women may have been limited, especially in the earlier studies, due to the smaller number of female alcoholic subjects in general (Dick and Bierut, 2006). The greater tendency for assortative mating among female than male substance abusers (and alcoholics) may actually cause greater familial loading (familial aggregation of drug disorders) among women (Merikangas et al., 1998).

In twin studies, addictions appear to be among the most heritable psychiatric disorders, based on correlations, which is not always the result of pure genetic influence, but may be due to gene-environment interactions and measurement errors (Goldman et al., 2005a). Drugs also differ in their addiction liability, which should correlate with heritability if the neurobiological basis of the addiction process was inherited. It seems that when using estimated risk rankings, the addiction liability predicts heritability, so cocaine and opiates, the most addictive substances, are among the most heritable. Cannabis, hallucinogens and caffeine are among the less addictive and less heritable. Alcohol has a medium risk of addiction development and also a medium degree of heritability (Goldman et al., 2005a; Bevilacqua and Goldman, 2009).

2.4.2 Molecular genetics studies

It is generally accepted that the risk for alcoholism is coded by multiple genes and polymorphisms in those genes, rather than a single "alcoholism gene". This is called polygenic inheritance, each gene (and its different allelic forms) exerting a small effect to reach a threshold of liability if combined with unfavourable environmental effects. On the other hand, genes may also be associated with protection from risk. There are two complementary methods in molecular approaches to identifying chromosomal regions and specific candidate genes: linkage and association. They both rely on the availability of polymorphic genetic markers throughout the genome. The polymorphic mutations in DNA may vary greatly in size, but the more common shorter mutations, involving only one or up to tens of bases, are the most useful as genetic markers for mapping the entire genome for research purposes. The two kinds of polymorphisms commonly used and listed in databases are variable number tandem repeats (VNTRs or microsatellites) containing multiple tandem repeats of two to five bases (the number of tandem repeats defining the allele), and single-nucleotide polymorphisms (SNPs), which are single-base substitutions at a particular position in a DNA sequence (most usually consisting of only two alleles at a locus). Several genetic marker sets that cover the entire human genome are commercially available (Dick and Foroud, 2002; Ball and Collier, 2002).

As a method, linkage can cover long genetic distances, but has low power to detect genes of small effect: it is usually able to identify only regions of interest on a particular chromosome. Conversely, association can detect genetic loci of minor effect, but is limited to short genetic distances. Systematic linkage analysis of the human genome has been feasible since the 1990s. The completion of the Human Genome Project and identification of a large number of single nucleotide polymorphisms (SNPs) spaced throughout the genome has made genome-wide association studies possible, rather than having to focus on chromosomal regions identified by traditional linkage method (Dick and Foroud, 2002; Ball and Collier, 2002; Quickfall and el-Guebaly, 2006).

2.4.2.1 Linkage to a chromosomal region

Linkage studies involve the comparison of affected and non-affected individuals within families or other large groups, with the intention of looking for genetic markers occurring with a higher rate than random distribution would allow. In other words, in family-based samples the purpose is to identify chromosome regions that are shared more often among phenotypically similar relatives (Sham and McGuffin, 2002).

One of the largest linkage studies on alcoholism is the Collaborative Study on the Genetics of Alcoholism (COGA), which collected detailed phenotypic data on individuals in families with multiple alcoholic members, starting in the middle of the 1990s. Initially a genome screen was completed in 105 multiplex alcohol-dependent families, and subsequently a replication sample of 157 similar independent alcoholic families. The control group included over 1200 individuals from different families (Reich et al., 1998; Quickfall and el-Guebaly, 2006).

The phenotype "alcohol dependence" (the clinical diagnosis) was linked to several chromosomes: 1, 2, 3, 4, 7, and 8. In addition, many other intermediate phenotypes (see below) created during the study were linked to chromosomes 4, 5, 6, 9, 13, 15, 16, 17 and 21. The phenotypes included some highly heritable electrophysiological measures reflecting central nervous disinhibition: abnormalities in EEG, evoked EEG rhythms and event-related potentials (such as increased betapower in resting EEG, reduced theta and delta oscillations, and a reduced P300 amplitude). As in complex diseases, the linkage regions were broad, encompassing hundreds of genes (Dick et al., 2006; Edenberg and Foroud, 2006).

After extensive genotyping and analysing, one of the most promising areas in the COGA sample seemed to be on chromosome 4p containing GABA receptor genes (GABRA2) (Edenberg et al., 2004). Several research groups replicated this finding (Lappalainen et al., 2005; Enoch et al., 2006b; Soyka et al., 2008). The adjacent gene GABRG1 has also been associated with alcohol disorders, and is suggested to contribute to the risk independently from GABRA2, in an additive manner (Covault et al., 2008; Enoch et al., 2009a; Ray and Hutchison,

2009). Thus these findings on both GABA receptor genes have already been replicated in several different population samples, including the F-N, GOGA, and some other cohorts. There were positive signals also on chromosome 4q containing alcohol dehydrogenase (ADH) gene cluster, and on chromosome 7q containing gene CHRM2 encoding muscarinic asetylcholine receptor subtype 2 (Edenberg and Foroud, 2006). A linkage signal was also derived from GABRA6 gene on chromosome 5 in the F-N cohort (Radel et al., 2005). However, these findings on chromosome 5 could not be replicated in the COGA cohort (Dick et al., 2005).

The COGA researchers stated that the major impact of the entire study was the finding that the linkage signals provided by the endophenotypes were more sharp (located directly over the gene subsequently found to be associated with the overlapping region of an endophenotype and clinical diagnosis of alcoholism). The clinical diagnosis alone did not necessarily yield a peak at the precise location (Dick et al., 2006).

One of the major limitations of the COGA was that even though the enrolled families were primarily of European descent, there was a considerable amount of heterogeneity. This was a potential confounder when searching for multiple genetic markers/genes with small effects (Quickfall and el-Guebaly, 2006). A second linkage study, also financed by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) like the COGA, was done in a much more homogenous population, a Southwest American Indian tribe. The study found one marker on chromosome 11 close to the D4 dopamine receptor gene and tyrosine hydroxylase genes, and on chromosome 4 close to GABA receptor genes (as in the COGA) (Long et al., 1998). Other large linkage study projects have been the Irish Affected Sibling Pair study (the Roscommon study), the Mission Indians (Native Americans) study, the study in a sample of multiplex families in the Pittsburgh area, and the studies in the Finnish F-N cohort (Prescott et al., 2005; Ehlers et al., 2004; Hill et al., 2004, Lappalainen et al., 1998). The advantage of isolated populations, and large families within them, is the reduced genetic heterogeneity. (In the model of heterogeneity, different

alleles lead to the same phenotype, but an individual allele can suffice to produce the phenotype.)

2.4.2.2 Association with an Identified Genetic Polymorphism

The frequency of linkage disequilibrium (LD) leads to indirect associations and false assumptions of causality, because the observed association may actually be at neighbouring allelic polymorphisms in LD with the studied allele. Another common reason for bias, population stratification, is caused by subgroups of the population in the study and the variance of the suspected allele frequencies and the disease frequencies between these subpopulations. One result of this is that we observe an association, either a false positive or a false negative, between an allele and a disorder in a subpopulation, without any actual causality (Sham and McGuffin, 2002).

The Hardy-Weinberg equilibrium or distribution (H-W) is used to define a pure random and independent transmission of alleles, which is violated if the genes are not picked freely from the pool. Assortative mating, most commonly inbreeding, biases the Hardy-Weinberg distribution. Population stratification is a form of hidden inbreeding (Gardno and McGuffin, 2002). Therefore, the more the allele frequencies differ from H-W equilibrium, the more likely the sample (and the results) are to be biased because of population stratification.

The simplest way to study association is by comparing individuals with the disorder/trait (cases) and unaffected subjects from the same population (controls) for differences in allele or genotype frequencies. There is an analogy to epidemiology, exposure here meaning the presence of an allele/genotype. In the most straightforward way, no family data of the subjects are required. If the real existing association is with a haplotype rather than a single gene, parental or even grandparental data are needed for a statistical procedure called haplotype relative risk calculation (HHRR) (Terwilliger and Ott, 1992). Briefly, a haplotype is a set of alleles at two or more adjacent loci, which are inherited together tightly linked on a segment of an ancestral chromosome generation after generation. HHRR compares the frequency of the two alleles passed from

the parents to the affected child with the frequency of the remaining two alleles. To avoid errors caused by population stratification, close relatives instead of unrelated controls are sometimes used (parents or sometimes unaffected siblings). In these family-based association tests, case-parent triads (the index subject with both parents, plus even siblings) may be used to compare the transmitted and non-transmitted alleles using the transmission/disequilibrium test (TDT) (Spielman et al., 1993). The transmitted alleles represent the case and the non-transmitted the control: the data are collected from a group of families. These two methods (HHRR and TDT) usually give similar results (Dick and Foroud, 2002; Sham and McGuffin, 2002).

2.4.2.3 Case-Control association studies on candidate genes – general issues and limitations

The development of molecular genetics led to the discovery of numerous polymorphisms, many of which were tested for an association with psychiatric disorders, especially from the beginning of the 1990s. Enthusiastic testing in different populations with samples of variable sizes led to conflicting results; the published association were usually soon followed by negative findings showing a lack of the suggested association between the polymorphism and alcoholism, for example. The negative linkage studies concerning candidate genes showing an association in case-control settings caused disbelief and confusion. The situation raised a lot of concern in the literature, even demands for a total discontinuation of case-control studies in genetics (Paterson, 1997).

Comings (1998) emphasized that each of the tested genes or polymorphisms could have explained only from 1% to 5% of the variance in alcoholism. In a polygenic disorder, at least 25 different genes, approximately, would be involved, and they can be very common in population. Consequently, polygenic disorders in different forms and degrees of severity are common, too. Negative linkage studies as well as negative HHRR and family-based association results should be expected concerning polygenic inheritance. They lose power in these situations, and exceptionally high numbers of subjects/families would be

needed. Comings also pointed out the importance of careful screening of the matched controls (matched for ethnicity as well, because the allele frequencies vary between these groups). The control group in a case-control study should be matched at least for age, sex, and ethnic and social background (as many environmental variables as possible), and above all else, for alcohol intake (Comings, 1998; Comings and Blum, 2000).

Trikalinos et al. (2004) scrutinized 55 cumulative meta-analyses of genetic associations (579 studies). Their purpose was to assess whether a statistical significance in early studies had any predictive ability for the outcome: established or refuted association after replications. The authors concluded that the magnitude of the effect in early studies could not adequately predict the true association. Conversely, many genuine associations would have been missed if the research had been abandoned as futile since the early underpowered studies showed negative results. The authors also reminded that biological plausibility supporting the association in the first observations, often emphasized in the literature, is not always straightforward and will not guarantee the future of the associations in the following replications. Biological reasoning can also be misleading if evoked *post hoc* to support the epidemiological findings (Trikalinos et al., 2004).

2.4.2.4 Intermediate phenotypes

The polygenic inheritance in behavioural traits (such as alcohol use) or in complex diseases (such as alcoholism) usually makes the impact and effect size of a single gene very small. If the whole diagnostic group of patients with complex disease/disorder is to be studied, extraordinarily large sample sizes are needed to detect the genetic loci with relevant but small effects on the phenotype. One promising approach to increase the power of association studies is to deconstruct the complex phenotype into disease risk mediating subunits, which are likely to be influenced by variation at fewer genes. These subunits can identify more homogenous clinical subgroups with common neurobiology and in some cases common genetic vulnerability, sometimes very

small subsets of the whole population of patients with a complex disease. These intermediate phenotypes are defined as mechanism-related manifestations of complex phenotypes. Sometimes they are almost synonymously referred to as endophenotypes. Strictly speaking though, an endophenotype is genetically inherited, and is an even smaller subset of the whole phenotype group than the intermediate phenotype including the risk mechanism of vulnerability. Intermediate phenotypes can be used to redefine the complex disease (Enoch et al., 2003; Schuckit et al., 2004; Goldman and Ducci, 2007). Gottesman and Gould (2003) descriptively define an intermediate phenotype as a "measurable component unseen by the unaided eye along the pathway between disease and distal genotype".

Heritability for alcoholism may be as high as 0.65 (Goldman et al., 2005a), so there is a need to identify more homogenous subpopulations sharing the mechanism of vulnerability among alcoholics, to direct attention to the particular genes responsible for aetiology. There already are intermediate phenotypes for alcoholism with respective genetic loci and polymorphisms. The involvement of these particular genetic polymorphisms was predicted on the basis of the known functional significance of the different alleles of these genes. Among these intermediate phenotypes and polymorphisms are: 1) attention/dyscontrol in cognitive performance tests and COMT Val158Met gene (with moderate heritability) and MAOA gene; 2) reward and DRD2 and OPRM1 genes; 3) stress/resiliency in performance tasks and questionnaires (such as TPQ) and 5-HTTLPR polymorphism in SLC6A4 gene (La, S, Lg alleles) and COMT Val158Met gene (with unknown degree of heritability); and 4) brain volume/structure assessed by MRI and several polymorphisms including 5-HTTLPR and COMT again (with heritability depending on the brain region) (Goldman and Ducci, 2007; Bevilacqua and Goldman, 2009). The level of response to alcohol (LR) is listed, too, with an estimated high heritability of 0.4–0.6. The genetic loci are not known, though, but some studies suggest an association between LR and 5-HTTLPR and some other gene polymorphisms, as discussed later in the section on this polymorphism and personality traits

(Enoch et al., 2003; Schuckit et al., 2004). Other intermediate phenotypes that have been suggested include deviations or abnormalities in EEG or event related potentials (ERP), lifetime history of depression, staying unaffected though living in an alcoholic environment, and maximum number of drinks ever consumed during a drinking session (Enoch et al., 2003).

2.4.2.5 Genome-wide scans and whole genome association

Genome-wide scans (GWS) include whole genome linkage, which was discussed above, and whole genome association (WGA) studies. Both allow a hypothesis-free mapping of suspicious loci within the genome. Linkage analysis is powerful for detecting the effects of uncommon alleles, whereas whole genome association detects the effects of relatively common alleles (present in over 5% of the study subjects) and gives a more refined localization in smaller chromosome regions. WGA analyses with dense panels containing more than 500 000 polymorphisms have been run in the search for associations in complex diseases, but the median odds ratios for one genotype have remained below 2. The typical effect size of genetic variations in complex diseases is small, and consequently the sample sizes required for a WGA study to reach the level of significance (0.05) has been estimated to be as high as 15 000 subjects. WGAs in large case-control data sets have not been reported for alcoholism (Ducci and Goldman, 2008).

WGAs lack the power to detect uncommon alleles, so they inevitably give false negative findings. But there is also a problem with false positive findings due to the high number of markers analysed, and multiple testing. Furthermore, current test panels are composed of SNPs only, while other types of polymorphisms, such as copy number variations (CNVs), should be interrogated to perform a genome-wide evaluation of suspicious disease loci (Ducci and Goldman, 2008). So far, 3 million SNPs have been listed, and they are estimated to cover 25–30% of all the relatively common SNPs in humans, with frequencies of over 0.05 in population. The number of loci involved in complex traits and diseases could be hundreds for many of them. The disease loci can

also have interactions with multiple linked genes, and each gene is is likely to contain multiple functional variants. Also, non-additive interactions can be present at all levels. Genetic complexity is present on multiple levels, and might be fruitfully thought of as fractal. Most probably there is a need to replace some current phenotypic and disease classifications with ones that better correspond to underlying genetic causes, by combining genotypic and phenotypic information (Kruglyak L, 2008).

2.4.3 Candidate genes showing association with alcohol use

Using complementary strategies of linkage and the candidate gene approach, several polymorphisms have been associated with alcohol use disorders. Most of the genes implicated have been related to neurotransmission, via neurotransmitter receptors or neuropeptides, or to alcohol metabolism, i.e. pharmacokinetics (Bevilacqua and Goldman, 2009). The most promising candidates according to the recent literature are briefly reviewed below. The biological hypothesis or support for the role of the particular gene or polymorphism in vulnerability to alcoholism is discussed. The studies on candidate genes of special interest in this study (COMT, 5-HTTLPR, TaqI A1) are reviewed last and more extensively. This is not meant to be – indeed, could not possibly be – a complete up-to-date list of the polymorphisms affecting the vulnerability to alcohol use disorders. On the other hand, the polymorphisms that may be involved in vulnerability to develop antisocial impulsive-violent type 2 alcoholism are discussed in a comprehensive way.

2.4.3.1 ADH and ALDH genes

Isoenzymes in the alcohol dehydrogenase (ADH) class play a major role in ethanol metabolism, converting alcohol to acetaldehyde. Polymorphisms in genes ADH1, ADH2 and ADH4 on chromosome 4 coding enzyme classes I, II and possibly IV are thought to be involved in individual differences in ethanol elimination. Acetaldehyde accumulation is responsible for the flushing and other aversive symptoms (nausea, headache, increased heart and respiratory rate).

(These actually are the effects of the relapse-preventing drug disulfiram.) The lower number of alleles ADH1B*2 and ADH1C*1 coding for more active enzyme forms have been associated with alcoholism, and these more active forms of enzymes leading to faster aversion are thought to be protective against alcoholism. The activities of the enzymes are different *in vitro* and *in vivo*, which has made the results of the association studies sometimes difficult to interpret. Different alleles of ADH1B gene seem to affect the risk and may protect from alcoholism, though the protective effect seems to vary across environments, so the impact is more or less inconsistent. The ADH4 gene has also been reported to be associated with alcoholism, but the role of allelic variants in the ADH2 gene remains uncertain (Oroszi and Goldman, 2004; Goldman et al., 2005a; Dick and Bierut, 2006; Quickfall and el-Guebaly, 2006; Higuchi et al., 2006).

After the conversion of ethanol to acetaldehyde, the next step is the conversion of acetaldehyde to acetate by aldehyde dehydrogenase (ALDH), primarily by ALDH2. The polymorphic gene coding for this isoenzyme goes by the same name and is located on chromosome 12. The enzyme produced by the ALDH2*2 allele is inactive, leading to aversive symptoms after alcohol ingestion, the impact being very strong among homozygous carriers. The allele is very common in Asians: a lower frequency of this allele was found among Japanese alcoholics as early as 1982. ALDH*2 most certainly plays a protective role by reducing the risk for alcoholism 10-fold, giving stronger protection than either ADH1B or ADH1C alleles. The effects of genes coding for high-activity ADH (faster production of acetaldehyde) and low-activity ALDH (slower elimination of acetaldehyde) are additive in producing protection against alcoholism. In general, the genes coding for alcohol-metabolising enzymes ADH and ALDH have been most consistently associated with alcohol dependence or protection against alcoholism (Goldman et al., 2005a; Dick and Bierut, 2006; Higuchi et al., 2006).

2.4.3.2 GABA receptor genes

$GABA_A$ receptors (i.e. type A) are the major inhibitory class of neurotransmitter receptors in the mammalian brain. (The other two types of GABA receptor superfamily are B and C.) They are activated by GABA, which is also the major inhibitory transmitter in the adult CNS. These receptors are also a target for benzodiazepines, barbiturates, anesthetics, neurosteroids and alcohol. They have been implicated in acute and chronic effects of alcohol, including tolerance, dependence and withdrawal (Krystal et al., 2006; Enoch et al., 2008b). GABAergic interneurons in the ventral tegmental area (VTA, which is part of the reward pathways in the human brain) act as inhibitory regulators of DA (dopamine) neurons. A subset of $GABA_A$ receptors in the VTA may be implicated in the switch from heavy drinking to dependence. $GABA_A$ receptors modulate anxiety and stress responses, the target symptoms of alcohol and benzodiazepine use, and benzodiazepines are commonly used to ameliorate alcohol withdrawal symptoms. In studies on knock-out mice lacking $GABA_A$ receptors, the most pervasive finding has been the decrease in alcohol consumption. $GABA_A$ receptors are composed of five heterogenous subunits (at least 21 subunits belonging to eight classes have been identified so far), allowing for tremendous diversity and multiple subtypes. These individual subunits of $GABA_A$ receptors have not yet been definitely linked with specific behavioural actions or effects of alcohol (Lobo and Harris, 2008; Enoch, 2008b).

Judging from the data on $GABA_A$ receptors and alcohol reviewed above, it is reasonable to search for genetic associations between these receptors and alcohol use disorders. Most of the genes coding for different types of $GABA_A$ receptors are organized into clusters on chromosomes 4, 5 and 15 (4p, 5q, 15q). The region containing $GABA_A$ receptor genes on chromosome 4 especially has consistently emerged in genome-wide linkage scans, with just the diagnosis of alcohol dependence, or even stronger when analysed together with electrophysiological (EEG) endophenotypes (Edenberg et al., 2004). A significant association was found with GABRA2 gene in that region. This finding was replicated later in case-control studies in different ethnic samples

(Lappalainen et al., 2005; Enoch et al., 2006b; Soyka et al., 2008). No functional polymorphisms affecting receptor function have been identified so far, so the evident and possibly even fundamental association lacks biological support. There have also been negative findings showing a lack of association between GABRA2 and alcoholism. On the other hand, there are results indicating that the association is with a haplotype rather than the single gene. It is difficult to draw definite conclusions after the finding that the two most abundant haplotypes in white study populations are both associated with alcoholism (Enoch et al., 2008b).

Recently, GABRG1, an adjacent gene to GABRA2, has been strongly suggested to predispose to alcohol use disorders. The effect is probably independent, but may be additive to the effect of GABRA2. The preliminary finding on this association (Enoch et al., 2009a) has already been replicated (Covault et al., 2008; Ray and Hutchison, 2009), which reflects the present interest in the association between GABA receptor genes and alcohol use disorders, and suggests that they might be important in future studies. There is also premilinary evidence of an association between GABA receptor genes (GABRA2) and externalising behaviour in childhood and adolescence, the suspected genetic effect being modified by environmental factors such as parental monitoring. In the innovative study by Dick et al. (2009) the gene-environment interaction (GxE) was represented, as intensified parental monitoring prevented the genetically predisposed vulnerable children from developing impulsive antisocial behaviours.

2.4.3.3 NPY, Galanin and GALR3 genes

Neuropeptide Y (NPY) is a neurotransmitter or a modulator consisting of 36 amino acids. It is believed to be involved in diverse biological functions: control of food intake, neuronal development, seizure activity, and emotional responses. In recent years NPY has been associated with neurobiological responses to ethanol and ethanol consumption or preference in general. Brain NPY levels have been shown to be inversely related to alcohol preference in

rodents. NPY-deficient knock-out mice drink more than wild-type mice, and transgenic mice over-expressing NPY drink less, and are also more sensitive to sedative/hypnotic effects of alcohol. The lower level of NPY in alcohol-preferring (P) rats compared with non-preferring (NP) rats has been explained by a functional polymorphism in the promoter region of the NPY gene on chromosome 7 (Oroszi and Goldman, 2004; Higuchi et al., 2006).

In humans, the expression of the Pro7 allele of the NPY gene might yield higher concentrations of blood NPY. This is uncertain, since the functional impact of the Leu(7) to Pro(7) polymorphism in humans is not known. The Pro7 allele has been linked to serious health risks: higher serum cholesterol levels in obese subjects, enhanced atherosclerosis, and higher blood pressure. It was also associated with a 34% higher ethanol consumption among middle-aged men in Eastern Finland, and with alcoholism among European Americans (Kauhanen et al., 2000). These findings could not be replicated in subsequent studies in Scandinavian populations. The later results even showed conflicting protective effects of the Pro 7 allele against alcoholism (Zhu et al., 2003). Again, a novel SNP of the NPY gene has shown an association with alcohol dependence, but this finding has not been replicated yet (Mottagui-Tabar et al., 2005). The data above show no definite role of the NPY gene in the development of human alcohol use disorders, though the studies in rodents were promising (Higuchi et al., 2006).

Galanine is a neuropeptide consisting of 30 amino acids, generated from preprogalanin, which in turn is encoded by the galanin gene (GAL) on chromosome 11q. Galanin is believed to regulate emotionality and anxiety in the limbic regions of the human brain. Animal studies have implicated galanin in alcohol abuse and anxiety. A study by Belfer et al. (2006) found a highly significant haplotype association, as well as a SNP association, between alcoholism and GAL among 514 Finns from the F-N cohort and 135 Plains American Indians (both samples included alcoholic and non-alcoholic males). The results suggest that a polymorphism in the GAL gene might contribute to vulnerability to alcoholism, possibly mediated by anxiety. The same research

group later reported an association between galanin receptor gene 3 (GALR3) and alcoholism. However, no functional polymorphism in galanin genes has been found yet (Belfer et al., 2006; Belfer et al., 2007).

2.4.3.4 OPRM1 gene

Opioidergic neurotransmission has been implicated in the reinforcing effects of several drugs of abuse, including alcohol and nicotine. Both ethanol and nicotine increase the level of the endogenous opioid β-endorphin (beta-) in a dose-dependent manner. The opioid receptor μ1 (OPRM1) is the primary site of action of β-endorphin (and the opioid receptor antagonist naltrexone). The effects of opioids in general are suggested to be mediated via at least three types of receptors, μ, κ, and δ (mu, kappa and delta). OPMR1s are widely distributed in the brain, the highest levels being found in the thalamus (mediating pain and stress responses), and in components of the limbic system, such as the amygdala, nucleus accumbens and gingulate cortex (mediating reward and emotions). β-endorphin release by alcohol mediates part of the rewarding effects of the drug either by stimulating OPRM1 or indirectly by releasing dopamine (in the reward pathways) (Oroszi and Goldman, 2004; Dick and Bierut, 2006). OPRM1 is encoded by the OPRM1 gene on chromosome 6 (6q), and it shows several polymorphisms. One is a common functional polymorphism Asn40Asp, first detected in the F-N cohort by Bergen et al. (1997; Anton et al., 2008). The less common Asp40 allele leads up to threefold higher binding affinity of β-endorphin at the receptor than the wildtype Asn40. The observed frequencies of the Asn40Asp alleles in humans have varied between 0.80-0.90 and 0.20-0.10. There were no differences in allele frequencies between violent alcoholic offenders and controls in the F-N cohort (Bergen et al., 1997).

Laboratory studies have suggested that the OPRM1 Asp40 allele might be associated with increased sensitivity to the effects of alcohol in humans, including feelings of euphoria (Higuchi et al., 2006). The drugs naltrexone and

nalmefene are associated with favourable but variable treatment outcomes in alcoholism. One or two copies of the Asp40 allele predicted lower relapse rates in a study by Anton et al. (2008), but there have also been contradictory findings (Arias et al., 2008). The influence of the OPRM1 gene on the development of alcohol dependence alone has remained obscure (Oroszi and Goldman, 2004; Dick and Bierut, 2006; Higuchi et al., 2006).

2.4.3.5 CHRM2 gene

A region on chromosome 7q was linked to the phenotype of alcohol dependence in the COGA study, and also to comorbid or independent diagnosis of depression, and to some electrophysiologic endophenotypes defined in the study. One of the identified genes in the region was CHRM2 coding for the muscarinic asetylcholine receptor subtype 2, which is involved in multiple brain functions. The finding was replicated in another study and sample. However, the case-control studies with SNPs of the gene have not shown any associations, nor have any functional polymorphisms been identified yet (Edenberg and Foroud, 2006; Higuchi et al., 2006; Dick and Bierut, 2006).

2.4.3.6 HTR1B, 5-HTR3, and MAO A genes

Serotonin (5-HT) and especially serotonin 1B receptor has been implicated in aggression in animal and human studies, as discussed above. Knock-out mice lacking the 5-HT 1B receptor gene (on chromosome 6q in humans) are more aggressive and have a preference for alcohol. This gene was linked to antisocial (type 2) alcoholism in the F-N cohort and in a southwestern Native American sample (Lappalainen et al., 1998). However, the replications have been variable, and no functional polymorphism has been identified (Goldman et al., 2005a).

The effect of serotonin at the 5-HT 3 receptor, consisting of subunits A and B, is directly potentiated by ethanol, and chronic alcohol intake sensitises the receptor (Enoch, 2003; Goldman et al., 2005b). Ondansetron, a 5-HT3 agonist, is used as an antiemetic. This drug has shown favourable treatment effects in

early-onset alcoholism, but a worsening of abuse problems in late-onset cases (Johnson et al., 2008). Ducci et al. (2009) discovered an association between a 5-HTR3B gene polymorphism (HTR3B AAG Ins/Del with estimated allele frequencies of 0.90 and 0.10), and also a haplotype involving that gene, and alcohol dependence combined with antisocial personality disorder in the F-N cohort. Enoch et al. (2009b) recently reported findings in the same F-N cohort suggesting that both 5-HT3A and 5-HT3B receptor genes might influence vulnerability to alcoholism, and a finding of a possible functional polymorphism, as well.

Monoamine oxidase A (MAO A) catabolizes serotonin, noradrenalin and dopamine. It is located on the X chromosome, which naturally causes the inherited single polymorphic allele effective in XY males. A common polymorphism in the MAO A gene's transcriptional control region ("MAOA-LPR") affects transcriptional activity, resulting in high and low activity alleleles. The impact of this polymorphism on serotonin metabolism is unclear, but it is presumed, on the basis of inconsistent data, that high activity of the MAO A enzyme leads to decreased transmission (Saito et al., 2002; Tikkanen et al., 2009). A prospective study by Caspi et al. (2002) with a large number of male children showed that negative childhood experiences and maltreatment together with low activity MAO A (possibly leading to higher than normal serotonin levels in the brain) predicted childhood conduct disorder and adult antisocial behavior. However, conflicting results of different studies have shown that both the high and low activity genotypes are associated with aggression and violence (Tikkanen et al., 2009). Tikkanen et al. (2009) showed that heavy drinking combined with MAO A high-activity genotype predicted recidivistic violence among 174 antisocial impulsive violent Finnish male offenders suffering from alcohol dependence or abuse, in other words from type 2 alcoholism. This was concluded to indirectly confirm the previous findings of the association between low CNS serotonin transmission and the risk of impulsive violence and type 2 alcoholism (Tikkanen et al., 2009). The role of the MAO A genotype in determining vulnerability to alcoholism alone has remained very

unclear judging from the inconsistent results of different studies (Caspi, 2002; Saito et al., 2002; Tikkanen et al., 2009).

2.4.3.7 Glutamatergic neurotransmission genes

The data concerning acute and long-term effects of alcohol on glutamate transmission, specifically on glutamate NMDA receptors, imply that genetic polymorphisms encoding glutamatergic neurotransmission might be involved in the development of alcohol dependence (Enoch, 2003; Schumann et al., 2008). Schumann et al. (2008) conducted the largest study on glutamatergic neurotransmission genes this far in southern Germany. Their samples consisted of 544 and 793 alcoholic subjects and 553 and 1002 controls, respectively in the first and second samples. The third sample consisted of 144 trios of 15-year-old adolescents and their parents, who were analysed for early risky drinking behaviour.

Based on the previous reports on the associations between glutamergic genes and alcoholism Schumann et al. (2008) chose 10 polymorphisms for a systematic analysis of alcohol dependence. The polymorphisms in genes NR2A and MGLUR5 (coding for NMDA receptor subunit 2A and metabotropic glutamate receptor 5) were shown to predict alcohol dependence in the first sample. In the second sample, they tried to independently replicate the finding, but only the NR2A polymorphism yielded a significant result. In the TDT test among 144 trios, overtransmission of the C allele of the NR2A polymorphism was observed, related to heavy drinking behaviour. The finding confirmed the impact of NR2A polymorphism, and possibly of NMDA receptors as well, in vulnerability to alcoholism, because early-adolescent alcohol abuse predicts alcohol related problems in adulthood (Schumann et al., 2008).

2.4.3.8 Other polymorphisms: HNMT, NTRK2 and CRH-BP genes

Histamine as a neurotransmitter has been studied far less than aminergic neurotransmitters, even though histamine is expressed in cortical and limbic areas of the human brain, which are involved in emotion and cognition.

The action of histamine-N-methyltransferase (HNMT) is crucial in terminating the neurotransmitter action of histamine. A C314T transition in exon 4 of the HNMT gene, which is a common functional polymorphism, results in a Thr105Ile substitution in the enzyme protein. The Thr105 allele has two-fold higher activity, probably predicting diminished histamine levels in the brain, and consequently may be involved in the individual differences affecting cognition and experienced anxiety or sedation. The allele frequencies of Thr105Ile are estimated to be 0.90/0.10 (Oroszi et al., 2005). In a large study of 857 subjects carried out among the Finnish F-N cohort and Plains American Indians, Oroszi et al. (2005) found the Thr105 allele to be more frequent among alcoholic individuals. The low levels of brain histamine in these subjects might be associated with higher levels of anxiety and might lead to self-medication with alcohol. On the other hand, varying levels of histamine may explain why some subjects are more resistant to the sedating effect of alcohol and are able to drink more than others. However, this finding of Oroszi et al. (2005) was not confirmed in a smaller sample of German alcoholics and controls (Reuter et al., 2007).

The role of brain-derived neurotrophic factor (BDNF) in the mechanisms of alcohol dependence has been demonstrated in animal studies. In humans, linkage scans of alcoholism have revealed chromosomal regions containing genes coding for BDNF and its cognate receptor, neurotrophic tyrosine kinase receptor B (NTRK2 gene). In a study among 500 Finnish antisocial alcoholics and controls (the F-N cohort), the entire NTRK2 region was covered with SNPs, three of which were associated with this subtype of alcoholism (Xu et al., 2007). This finding concerning brain growth factors is of interest considering the results of a brain imaging study showing increased white matter in the brains of violent antisocial alcoholic subjects. However, this quite unexpected observation by Tiihonen et al. (2008) has not yet been replicated.

The resting EEG is a dynamic index of cortical activation, cognition and consciousness and has been suggested to be heritable. Enoch et al. (2008a) used resting EEG frequency bands (alpha, theta and beta) as intermediate

phenotypes in a dense whole genome linkage scan among 300 Plains Indians and 200 white Americans. They found a connection between the resting EEG power, anxiety, alcohol use disorders and the CRH-BP gene coding for high affinity binding protein for corticotrophin releasing hormone (CRH). The binding protein modulates the action of CRH, a primary mediator of the mammalian neuroendocrine and behavioural responses to stress, which makes CRH-BP a relevant candidate gene for anxiety and addiction (Enoch et al., 2008a).

2.4.4 Association studies on COMT gene polymorphism

2.4.4.1 COMT enzyme and COMT polymorphism

Catechol-O-methyltransferase enzyme inactivates catechol hormones, drugs containing catechol (e.g. L-dopa for Parkinson's disease) and catecholamines, including those acting as neurotransmitters such as dopamine. COMT enzyme occurs in mammals as two distinct forms: a cytoplasm-soluble protein (S-COMT) and a membrane-bound protein (MB-COMT). S-COMT activity is the most prevalent in all tissues except the human brain, where most of the enzyme activity (70%) comes from MB-COMT (Tenhunen et al., 1994; Syvänen et al., 1997). The level of COMT activity in humans has been shown to vary between individuals in trimodal distribution of low, intermediate and high levels, and was known to be genetically polymorphic already in the 1970s (Lachman et al., 1996).

Both forms of COMT enzyme are coded by a single gene, which is expressed in all tissues and located on chromosome 22q11. No brain specific variants have been detected (Syvänen et al., 1997; Lachman et al., 1996). A common polymorphism at codon 108/158 (S-COMT / MB-COMT) of the COMT gene, resulting from a nucleotide transition (G to A) and causing a valine to methionine substitution in the enzyme, was found to be functional. Homozygosity for the valine (G) allele was associated with three- to four-fold enzyme activity compared with the homozygous methionine (A) genotype (and thus possibly leading to less sustained dopamine effect in the brain). The

heterozygous genotype had an intermediate level of enzyme activity. At least some of the variation in activity may be caused by the thermolability of the COMT-methionine form. These results obtained from human liver tissue or red blood cells will very probably be expressed in brain tissue as well (Lachman et al., 1996). The frequency of the valine/methionine alleles in white population is found to be 0.51/0.49 (Bevilacqua and Goldman, 2009). The COMT valine and methionine alleles (val and met) are later also referred to as H (for high activity) and L (for low activity) alleles, respectively.

The sex- and brain-region-specific contribution of COMT enzyme activity (of genetic origin) in the maintenance of dopamine levels and in the regulation of dopamine neurotransmission and consequent changes in behaviour was shown in a study on COMT knock-out mice (Gogos et al., 1998). COMT-deficient homozygous male mice showed a 2- to 3-fold higher level of dopamine in the frontal cortex, but no changes of dopamine content in either the striatum or hypothalamus were noted, nor were there any locomotor deficits present. COMT-deficient homozygous female mice showed higher anxiety. Aggressive behaviour was markedly higher in heterozygous COMT-deficient male mice. The authors speculated that the COMT activity of a heterozygous COMT-deficient mouse is comparable with that in a human subject homozygous for the L allele, although in general experimental results on knock-out mice cannot readily be used as a model for complex human behaviours.

It has been detected that COMT valine and methionine alleles reside on haplotypes (i.e. in patterns of alleles at nearby linked loci, transmitted and inherited together rather than segregated by meiotic recombination), and these haplotypes are common to different populations. These aspects reflect an ancient origin of both alleles in the human genome, and a potential role of selection in their maintenance (Oroszi and Goldman, 2004; Goldman et al., 2005a).

2.4.4.2 COMT polymorphism and cognitive function, and personality traits

The abundant literature concerning COMT polymorphism and major mental disorders such as schizophrenia or bipolar disorder is not discussed here because it is irrelevant. The effect of COMT polymorphism on personality and cognition is closely related to alcohol use disorders, and it is therefore briefly discussed below.

The inherited differences in COMT activity are likely to predict variation in dopaminergic neurotransmission in the frontal cortex of the brain (prefrontal area), where the levels of another crucial regulator, dopamine transporters, are low. This was shown in the study on COMT knock-out male mice described above (Gogos et al., 1998). Solid data indicate that dopamine enhances prefrontal neuronal function during working memory tasks and thus also enhances cognitive performance (Egan et al., 2001; Goldman et al., 2005a).

A highly sophisticated study among 175 patients with schizophrenia, their 219 unaffected siblings and 55 healthy controls showed that low activity L allele of COMT polymorphism predicted better cognitive performance during neuropsychological testing of executive functions. When the normal subjects were assessed with functional magnetic resonance imaging (fMRI) during a working memory task, the L allele again predicted a more efficient physiological response in the prefrontal cortex. The effect was allele dose dependent: the individuals homozygous for L allele showed the most efficient results, and heterozygotes were intermediate when compared with the least efficient group of subjects homozygous for high activity H allele (with faster dopamine inactivation and less sustained transmission). In a family-based analysis there was also a significant hereditary transmission of the H allele to schizophrenic offspring. This indicates that the COMT H allele might also be if not an actual risk factor for then a modulator of the prognosis in schizophrenia, because it impairs prefrontal function (Egan et al., 2001). The COMT H allele and its haplotypes have been linked to prefrontal lobe function in other studies, as well (Malhotra et al., 2002).

On the other hand, the low activity L allele has also been linked to higher levels of anxiety and a lower pain threshold. A study among two community samples of 149 white and 252 Plains American Indians subjects (predominantly females, without lifetime psychiatric diagnosis) found an association between COMT L/L homozygous genotype and higher anxiety levels measured by the Tridimensional Personality Questionnaire (TPQ, Cloninger et al., 1991) and low-voltage alpha resting EEG among the female subjects (Enoch et al., 2006a). These EEG findings had previously been shown to be associated with anxiety and alcoholism by the authors, potentially reflecting intermediate mechanisms (Enoch et al., 1999). A lower pain threshold, stronger affective response to pain, and inability to activate the endogenous brain opioid system following pain was associated with the L allele in a brain imaging study by Zubieta et al. (2003). Lower pain threshold was associated with L allele haplotypes in a study of a large cohort of women by Diatchenko et al. (2005), as well.

Women generally are considered to be more prone to anxiety than men. However, the findings above might indicate that the carriers of the low activity L allele tend to "worry" or suffer from anxiety because of the lower resiliency to stress and pain, but they also gain better cognitive functions in change (with more sustained frontal dopamine transmission). This balance of advantages forms the basis for the "Warrior/Worrier" model (Goldman et al., 2005a): the warrior has better stress resiliency, and a higher pain threshold with a more functional endogenous opioid system, but also less effective executive cognitive performance (which probably reduces stress before the “battle”). This dichotomy of advantages/losses may be responsible for the selection to conserve both of these alleles (or haplotypes containing the alleles) in the human genome across populations.

There are also differences in COMT activity based on sex. At least in vitro estrogen can inhibit COMT gene transcription, and women have been shown to have significantly lower COMT activity than men (Enoch et al., 2006a). This is consistent with the data mentioned above on the association between anxiety and low COMT activity and, on the other hand, between female sex and

predisposition to anxiety. Sexually dimorphic behavioural changes in mice associated with low COMT activity are described above (Gogos et al., 1998). Altogether, this indicates that sex may modify genetically determined behaviour, the same genotype resulting in different behavioural consequences in males and females.

It has also been suggested that the COMT genotype and associated variation in prefrontal cognitive function act as a modifier against a background of other aetiological factors to affect behaviour. In a recent study among almost 500 children from three cohorts, Caspi et al. (2008) showed a conditional association between antisocial conduct disorder and the COMT H/H (Val/Val) genotype, presumably with less effective prefrontal cognitive processing. The association was observed only in children with ADHD, not in those without. It is generally accepted that about 50% of the children and adolescents with ADHD show antisocial tendencies (Caspi et al., 2008).

2.4.4.3 COMT polymorphism and alcoholism or substance abuse

Dopamine release in the human brain has been suggested to play a major role in euphoria induced by substances of abuse and thus possibly in the development of alcoholism. Consequently, it would be logical to search for an association between the functional COMT L/H polymorphism and alcoholism or other substance abuse. A study among 175 Japanese alcoholics (the majority males) and 350 age and gender matched controls found no difference in COMT allele frequencies or genotypes between subjects and controls. No association between genotypes and age of onset of alcoholism or antisocial behaviour were detected, either (Ishiguro et al., 1999a). Vandenbergh et al. (1997) found that the COMT H allele was significantly more common among 200 white polysubstance abusers than 100 controls. The association was explained through impulsivity or dyscontrol, not by liability to excessive anxiety. Altogether, association studies on the COMT genotype and alcoholism were rare in the 1990s.

2.4.5 Association studies on serotonin transporter gene polymorphisms

2.4.5.1 Serotonin transporter (5-HTT) and 5-HTT gene polymorphisms

Serotonin transporter (5-HTT) is a functional protein that regulates the amount of serotonin (5-HT) in a synaptic gap by taking up 5-HT back to a presynaptic neuron and hence finishing transmission. It is therefore crucial in fine-tuning brain serotonergic neurotransmission controlling the magnitude and duration of 5-HT responses (Lesch et al., 1994; Lesch et al., 1996). 5-HT and its transporter are abundant in the central and peripheral nervous system, and are also found in platelets, placental and pulmonary plasma membranes.

The human 5-HTT is encoded by a single gene (termed SLC6A4) on chromosome 17 (more precisely 17q11.1-17q12) (Lesch et al., 1994; Lesch et al., 1996). A variable number tandem repeat polymorphism (VNTR) is found in the second intron of the gene, with at least four different alleles identified as containing 9–12 repeats (Lesch et al., 1994). The functional impact of the VNTR polymorphism remains unknown.

Another well-known polymorphism is located 1 kb (kilobases) upstream from the transcription site in the promoter region of the 5-HT gene (in the 5´- flanking region) and is known as 5-HTTLPR (5-HTT gene linked polymorphic region) or the SERT gene (Lesch et al., 1994; Lesch et al., 1996). The polymorphism consists of 44-base pair insertion- or deletion-involving repeat elements. The alleles are called L (long) and S (short) depending on the number of repeats. The polymorphism appears to influence gene function by changing transcription efficiency. The basal activity of the L variant is more than twice that of the S form. That means that 5-HT uptake in cells homozygous for L allele is twice that in cells carrying one or two S alleles. It also suggests a dominant-recessive nature of the polymorphism with possible dominance of the S allele (Lesch et al., 1996), but this has subsequently been questioned, and a co-dominant action of the alleles has been proposed (Hu et al., 2004). A study using 5-HTTLPR knock-out mice to reduce transporter expression demonstrated a marked dose-dependent increase in extraneuronal 5-HT in the striatum and

frontal cortex, associated with a decreased expression of the gene (Mathews et al., 2004).

Later, 14 new variants were found among Japanese and white subjects, both for S type and for L type, with proposed but not observed functional differences between them (Nakamura et al., 2000). Hu et al. (2006) showed the functionally triallelic nature of 5-HTTLPR polymorphism: a common single-base substitution (A to G) in the L allele creates La and Lg alleles. In lymphoblastoid cell lines (in vitro) the transcriptional activity of the Lg is reduced to the same level as the S allele (Hu et al., 2006). Hu et al. (2006) speculated that the main result of not paying attention to the triallelic nature of 5-HTTLPR, i.e. not scoring the low function Lg allele, is probably to obscure the effect of the highest expressing LaLa genotype. The effect is perhaps less crucial for phenotypes previously associated with the low activity S allele. The authors listed the frequencies of these three alleles, and the six new functional genotypes in different populations. In a group of 770 Finns the frequencies were: Lg allele 0.09, SLg genotype 0.08, LgLg genotype 0.1. (Consequently, the net effect would be a frequency of +0.09 added to the SS genotype group mainly from the former SL group.) The Lg allele frequencies varied considerably between populations, being highest among 600 African Americans (0.24) and lowest among American Indians (0.01) (Hu et al., 2006). However, other researchers have not been able to replicate the results of Hu et al. (2006) regarding the association between Lg allele and lower 5-HTT expression (Martin et al., 2007).

The studies on 5-HTTLPR polymorphism reviewed below, despite the confusing data by Hu et al. (2006), are based on the findings by Lesch et al. (1996) of the biallelic polymorphism and the functional difference in transcriptional activity between L and S type alleles. The frequency of the L and S alleles among white subjects is 0.60/0.40 respectively. If the low activity Lg allele is included, the allele frequencies L/S/Lg are 0.50/0.40/0.10. Even after the report by Hu et al. (2006) it is generally claimed that the 5-HTTLPR gene has only two common functional alleles: L and S. The in vivo transcriptional

effects of the single-nucleotide polymorphisms described by Hu et al. (2006) are not known (Uher and McGuffin, 2008).

2.4.5.2 5-HTTLPR and personality related traits

The abundant literature concerning 5-HTTLPR polymorphism and bipolar disorder, especially depression and the suggested GxE interactions, is not discussed here. It is not relevant, whereas the effect of the 5-HTTLPR polymorphism on personality is closely related to alcohol use disorders, and is briefly discussed below.

In addition to testing possible associations between actual psychiatric disorders and known polymorphisms, a substantial body of studies has examined the relationship of candidate genes to personality dimensions. However, the results have been inconsistent. The polymorphisms most often studied are presumed to be involved in the regulation of the serotonin- or dopamine-mediated neurotransmission. A meta-analysis by Munafò et al. (2003) listed 46 studies reporting data on associations between candidate genes and human personality traits, screened by different personality scales, most often with NEO inventories (Costa and McCrae, 1997) or TCI/TPQ personality inventories (Cloninger et al., 1991). Studies involving subjects with psychiatric disorders such as substance abuse were excluded. In a fixed-effects model, significant associations were found related to serotonin transporter (5-HTTLPR) and dopamine receptor (DRD2, DRD3 and DRD4) polymorphisms. Half of the studies reported data on 5-HTTLPR. After multivariate analysis to control for the significant heterogeneity between the studies, only the association between the 5 HTTLPR polymorphism and so-called avoidance traits (but not aggression or approach traits, which were also studied) remained significant. However, even that significance was lost after further sensitivity analyses (excluding studies with allele frequencies not in H-W equilibrium) (Munafò et al., 2003).

A more recent meta-analysis concentrating on 5-HTTLPR polymorphism and anxiety-related personality traits in adults (Sen et al., 2004) gathered data on 23

studies involving 5629 subjects. The first published association between 5-HTTLPR and neuroticism, screened by the NEO inventory, was reported by Lesch et al. (1996). Neuroticism is characterized as "negative emotionality", such as anxiety, low mood, vulnerability and hostility. Roughly half of the studies included used NEO, measuring neuroticism, and the rest used the TCI/TPQ scale, measuring harm avoidance. These two traits have a correlation of 0.55 (De Fruyt et al., 2000), indicating significant variation in the outcome measures by the two scales. The meta-analysis shows a borderline significant association between the 5-HTTLPR S allele and increased anxiety as a trait (P=0.087). Eight studies of the 23 listed found a significant association. Not a single study found an association between the L allele and higher anxiety trait scores. This argues against a chance finding in an overall analysis. There was a significant heterogeneity among the results of the different studies also in this meta-analysis, but it was more limited among studies using NEO. The demographic variables such as gender and ethnic composition were not significant confounding variables (Sen et al., 2004).

Studies of the association between 5-HTTLPR and physical or verbal aggression, reflecting behavioural disinhibition as a personality trait, have been scarce. A Chinese study in Taiwan (Liao et al., 2004) among 135 males convicted for extremely violent crimes found an excess of S allele among index subjects compared with 111 controls, but no association between ASP or substance abuse. Haberstick et al. (2006) conducted family-based tests of association in a sample of 732 twins and their parents. They found a borderline significant association between S allele and aggressive behavior in middle childhood (at the age of 9).

An elegant study among a relatively small number (28) of healthy subjects but using a direct assay of brain function, BOLD fMRI (blood oxygen level dependent functional magnetic resonance imaging), studied the association between 5-HTTLPR polymorphism and amygdala response to fearful stimuli (Hariri et al., 2002). Based on the previous studies showing abnormal levels of anxiety among S allele carriers, and the recognized role of amygdala in 5-HT-

mediated fear responses, the authors presumed that individuals with the S allele would exhibit a greater amygdala response. The genotype groups did not differ in overall performance, indicating a lack of non-specific effects on the results. Neither were there significant group differences in anxiety- or fear-related traits screened by the standard behavioural measure TPQ (Tridimensional Personality Questionnaire, see above), probably because of the small sample size. The main results, however, confirmed the authors‘ hypothesis: the responses of the right amygdala were significantly greater among the S carriers than among subjects homozygous for the L allele. The heightened responses probably reflect increased excitatory action of synaptic 5-HT due to the decreased 5-HT transporter expression in S allele carriers, but may also reflect partial desensitization of inhibitory 5-HT1A receptors following high levels of synaptic 5-HT. Finally the authors conclude that the observed different responses in adult subjects may be rooted in early individual developmental processes influenced by serotonergic neurotransmission.

In this context, the level of subjective response to alcohol’s desired (intoxicating, euphoric) or negative (e.g., nausea) effects are viewed as a personality trait. "Low Level of Response (low LR)" is also one of the more thoroughly investigated phenotypes associated with alcoholism risk (Schuckit et al., 2004). It is considered to be a risk-related, inherited intermediate phenotype possibly preceding alcoholism, as described above. One of the polymorphisms studied for an association is 5-HTTLPR. Schuckit et al. (1999) published a follow-up case-control study in 1999, and later they expanded it by adding more subjects, for a total of 85 males with clearly high or low LR to alcohol. The 5-HTTLPR L allele was associated with low LR at age 20 and with the development of alcohol dependence or abuse during the follow-up of 10, 15 and 20 years (Schuckit et al., 1999; Hu et al., 2005). Also, the polymorphism called GABA A alpha6 Pro385Ser in chromosome 5 and the uncommon 385Ser allele showed a non-significant trend for association to a low LR. The 5-HTTLPR L allele showed an allele-dosage (stepwise) association with LR and also alcoholism, and the effect of GABA A alpha6 Pro385Ser was additive to that

(Hu et al., 2005). The authors also subdivided L alleles into functional subtypes La and Lg, without a change in the observed association.

2.4.5.3 5-HTTLPR polymorphism (S and L alleles) and alcoholism

Central nervous serotonin activity has been shown to modulate alcohol consumption in animals (LeMarquand et al., 1994). This has encouraged the study of the 5-HTTLPR gene as a potential candidate in alcoholism. An association between the S allele and severe dependence was detected in a subset of 100 subjects drawn from a sample of 315 German alcoholics (Sander et al., 1997). A trend towards association between the S allele and alcoholism was observed in a smaller subset of 64 antisocial subjects from the same sample (Sander et al., 1998). Also, Hammoumi et al. (1999) detected an excess of the S allele among French alcoholics. Ishiguro et al. (1999b) found an association between the L allele and earlier onset of dependence among 166 Japanese alcoholics and 290 controls. Negative findings were reported in two studies among European American subjects (Gelernter et al., 1997; Edenberg et al., 1998a). Jorm et al. (1998) also failed to find any association between 5-HTTLPR genotypes and alcohol disorders in a large white Australian general population sample of 759 subjects.

2.4.6 Association studies on dopamine receptor D2 gene polymorphisms

2.4.6.1 DRD2 and DRD2 gene polymorphisms

The dopamine receptor D2 (DRD2) belongs to a D2-like family of the human dopamine receptors together with the receptor subtypes D3 and D4. The other family includes receptor subtypes D1 and D5. The gene for D2 is isolated and sequenced: it is located on chromosome 11 at q22-q23 (Grandy et al., 1989). Several polymorphic markers have been identified in the DRD2 gene, the review by Pato et al. (1993) already listed 8 identified polymorphisms in this gene. Among them are Ser311Cys and –141C Ins/Del, the latter being a functional polymorphism. The allele frequencies of the –141C Ins/Del are

0.91/0.09 (Bevilacqua and Goldman, 2009). However, our knowledge of how the transcription of the human D2 receptor is regulated is limited (Pohjalainen et al., 1998; Parsian et al., 2000). In the literature none of the several polymorphisms located in the coding region of the DRD2 gene (thus more possibly affecting the transcription) shows a strong replicated association with psychiatric disorders.

The TaqI A polymorphism, a RFLP (restriction fragment length polymorphism) with two alleles called A1 and A2, was the first of the DRD2 polymorphisms to be detected. It has been the most studied, the DRD2 gene in general probably being the most intensively studied gene in alcoholism during the 1990s (Noble 1998a). (During this decade the most studied topic was probably the genetics involved in GABA transmission, especially in the GABA receptors.) The results concerning associations between TaqI A and alcoholism (Blum et al., 1990; Noble et al., 1991; Noble 1998a), or the lack of associations have been controversial (Bolos et al., 1991; Goldman et al., 1992; Gelernter et al., 1993). Unfortunately the polymorphic TaqI A site is located outside the coding region of the DRD2 gene (10 kb downstream from the coding region). Consequently, it would have to be in linkage disequilibrium with another clearly functional polymorphism to affect D2 receptor availability, and possibly the development of individual personality traits or vulnerability to psychiatric disorders (Laruelle et al., 1998). However, there is evidence of a TaqI A SNP and linkage disquilibrium involving TaqI A that extends into the DRD2 gene, up to 25 kb away from the TaqI A itself. In other words, the possibility of LD between TaqI A and DRD2 is not totally excluded (Kidd et al., 1998).

Recently it has been recognized that the TaqI A polymorphism is actually located within the coding region of a kinase gene neighbouring the DRD2 gene. This complex, called the ankyrin repeat and kinase domain, contains the ANKK1 gene, and probably codes for proteins involved in signal transduction pathways. ANKK1 is a novel serine/threonine kinase gene, one of the "tyrosine kinase like genes (TKL)" (Neville et al., 2004). It is suggested that TaqI A SNP polymorphism actually causes an amino acid substitution within the substrate

binding domain of this gene, affecting binding specificity. In this way TaqI A polymorphism could be involved in dopaminergic transmission along with ANKK1, via a signal transduction pathway or other cellular responses to external stimuli. Genes of related functions (such as DRD2 and ANKK1) are sometimes found clustered together (Neville et al., 2004; Hill et al., 2008). There is some preliminary evidence from the COGA sample suggesting an association between SNPs in the DRD2/ANKK1 and severe alcohol dependence (Dick and Bierut, 2006).

Also, a recently identified SNP of the DRD2 gene, C957T, has been proposed as being responsible for some of the phenotypic features previously associated with the TaqI A polymorphism, such as D2 receptor availability in the striatum (Hirvonen et al., 2004). On the other hand, the TaqI A of the ANKK1 gene is associated with increased striatal activity of aromatic L-amino acid decarboxylase, which is the final enzyme in the biosynthesis of dopamine (Laakso et al., 2005). C957T and TaqI A polymorphisms have been reported to be in LD with each other, and both have been suggested to have a functional impact in dopaminergic transmission. It is unclear which of the previously reported associations with TaqI A are explained by associations with ANKK1 and which by associations with C957T (Ponce G et al., 2008).

TaqI B, C and D polymorphisms have also been identified. TaqI B is supposed to be in strong linkage disequilibrium with TaqI A, but the functional role of any of the TaqI polymorphisms is not clear (Ritchie and Noble, 2003).

2.4.6.2 TaqI A polymorphism and DRD2 availability

After Blum et al. (1990) discovered the association between TaqI A and alcoholism, Noble et al. (1991) published their finding on reduced DRD2 binding capacity associated with the TaqI A1 allele in a dose-dependent fashion. The A1 allele was also strongly associated with alcoholism in this same study. The brain samples of 66 alcoholic and non-alcoholic subjects were studied post-mortem. Also, Thompson et al. (1997) found an association between the A1 allele and reduced DRD2 receptor binding in the human striatum. They studied

44 healthy middle-aged to elderly subjects post-mortem by autoradiography. Pohjalainen et al. (1998) studied 54 healthy Finnish volunteers with positron emission tomography (PET), and found a statistically significant reduction in DRD2 availability in subjects with the A1/A2 genotype compared with the A2/A2 group (the study group did not include any A1/A1 subjects). The existence of the A1 allele accounted for 12% of the variance in DRD2 availability. Jönsson et al. (1999) obtained similar results, an association between A1 allele and low DRD2 density, in their PET study on 56 healthy white subjects, but the sample included only a few A1/A1 subjects. In a post-mortem study, Ritchie and Noble (2003) found a lower number of DRD2 binding sites in the brains of A1 allele carrier subjects (40% fewer receptors compared with those without A1). This was a subset of their former 1991 study involving brain tissue from 20 alcoholic and 21 nonalcoholic subjects. Noble et al. (1997) also studied mean relative glucose metabolic rate (GMR) by PET in different brain regions of 15 healthy white volunteers while they were executing a continuous performance task. They found lower GMR in dopamine receptor-rich brain areas, closely associated with the prefrontal system, in subjects carrying A1 allele.

In some of the above studies an association between the TaqI B genotype and DRD2 availability was also found, which seems to be quite natural considering the replicated finding of a strong linkage disequilibrium between TaqI A and TaqI B polymorphisms. One of the so-called synonymous ("silent") mutations, C957T mentioned above, has been shown to explain 18% of the variance in striatal binding potential (BP) (Hirvonen et al., 2004). Together with the effect of the TaqI A1 allele, they explained 40% of the variance in DRD2 BP, in other words, receptor availability.

There are also conflicting findings. Laruelle et al. (1998) studied 47 healthy controls and 23 subjects with schizophrenia (and with previous exposure to dopaminergic antipsychotic drugs) by PET. The study group, none of the subjects with a history of alcohol or other substance abuse, included a few subjects with the A1/A1 genotype. They could not find any association between DRD2 binding potential and TaqI A or B genotypes. Commenting on the

conflicting findings, Hitzemann (1998) pointed out that in previous studies the frequency of the A1 allele had varied considerably in subjects from different ethnic groups, which explained some of the variance in the results. Moreover, the age of the study subjects had not been controlled for, and aging seems to cause a marked receptor loss in animals with high youthful receptor density. Previous studies had strongly indicated that DRD2 density/availability is regulated by multiple genes, not just the DRD2 gene, which would likely have only a moderate effect size, other genes controlling most of the variance. There is also a wide natural variation in DRD2 density among normal controls without any neurological or psychiatric pathology (Hitzemann, 1998). Gelernter et al. (1993) had already emphasized the importance of controlling for the ethnic origin of the study samples when they were criticizing the first over-optimistic observations on the association between TaqI A polymorphism and alcoholism.

To clarify the subtype of the dopamine receptors involved in alcohol self-administration, Phillips et al. (1998) studied DRD2 knock-out mice. These mice showed an aversion to alcohol and were less sensitive to movement disorders caused by alcohol than wild-type mice. Homozygous D2 -/- mice lacking D2 receptors consumed only half the amount of alcohol that the wild-type mice consumed, and heterozygous mice had an intermediate level of alcohol consumption. Referring to the debate about the association between alcoholism and DRD2 polymorphism, the authors stated that their study neither supported nor refuted the influence of allelic variation at the human DRD2 locus in modulating ethanol consumption. "Whether naturally occurring DRD2 allelic variation could account for large differences in ethanol preference drinking and sensitivity in mice, similar to those induced by deletion of the DRD2 gene, remains to be established", the authors concluded.

2.4.6.3 TaqI A polymorphism and personality traits

The aetiology of alcoholism has been associated with several behavioral markers in children of alcoholics, such as cognitive deficits in visuospatial ability. Connected with this, alterations in brain electrical potentials have been

observed in these children, such as reduced amplitude and prolonged latency of the P300 event-related potential (ERP). Many studies have shown that children raised by alcoholic parents experience significantly more stressors than usual, and this probably makes them more vulnerable to alcohol use disorders. Based on these findings, in a partial replication of their earlier study, Berman et al. (2003) studied 146 healthy high-risk sons and daughters of alcoholic parents. The somewhat confounding results indicated that both sex and TaqI A genotype modified the association between negative affect and other risk markers. Among the TaqI A1 allele carrier boys, experienced family stress was associated with reduced visuospatial ability and reduced P300 amplitude.

Madrid et al. (2001) tested the predictive power of the TaqI A genotype in the development of alcoholism in a sample of 309 males of Mayan descent in Honduras. The participants were interviewed and then more systematically studied for alcoholism and stress and for TaqI A genotypes. (The authors remarked that 20% of the working population in Honduras suffers from alcoholism.) Three explanatory models for alcoholism were tested: 1) demographic variables alone; 2) stress and TaqI A genotype separately; 3) interaction between stress and genotype. Neither alcoholism nor experienced stress alone was associated with genotypes. However, stress and the Taq A1 allele together significantly predicted the degree of alcoholism (model 3). Stress had no effect on the development of alcoholism in the A2/A2 genotype group and only a modest effect among heterozygous A1/A2 subjects. The authors suggested that this was further evidence of gene-environment interaction, and also that this could explain the variance in the results of previous studies on alcoholism and TaqI A genotypes, since they had not accounted for experienced stress (Madrid et al., 2001).

In a study by Ponce et al. (2003) among 103 alcohol-dependent Spanish males, subjects with the TaqI A1 allele had a higher prevalence of antisocial personality disorder and more often had a family history of alcoholism. A study by Limosin et al. (2003) among 92 French alcoholics (both sexes) showed that

the A1/A1 genotype was associated with lower impulsiveness assessed by Barrat's Impulsiveness Scale.

A systematic review and meta-analysis of genetic polymorphisms and personality in healthy adults by Munafo et al. (2003) of 46 studies on different candidate genes found six studies on TaqI A polymorphism and personality traits (involving 600 study subjects). There was evidence of a significant association between the TaqI A genotype and so-called "avoidance traits", but the significance was lost when the heterogeneity between the studies was controlled for in a random effects model. In other words, there was no consistent association between TaqI A polymorphism and adult personality traits on a meta-analysis level.

2.4.6.4 TaqI A polymorphism and smoking

Alcoholism and smoking are highly comorbid, with 80–90% of alcohol-dependent subjects smoking (three times more than in the general population). There is evidence of shared as well as for specific addiction vulnerability between these behaviours (cross-inheritance and independent heritance). There also seems to be a considerable genetic overlap between these addictions particularly in the group of heavy drinkers/heavy smokers. The nicotine in tobacco smoke is known to be a dopaminergic drug affecting the reward pathways (Enoch, 2003). Consequently, the DRD2 gene and TaqI A polymorphism have also been studied among smokers. Comings et al. (1996) studied 312 white heavy smokers from a cessation clinic and 700 controls, finding a significant association between the A1 allele and smoking, young age of onset of smoking and unsuccessful attempts to quit smoking. Spitz et al. (1998) studied 157 lung cancer patients and 126 controls but could not find any associations between TaqI A genotypes and smoking. Instead, the TaqI B1 allele was associated with smoking, and the prevalence of the rare A1 allele seemed to predict the presence of the equally rare B1 allele (linkage disequilibrium). These few studies attempting to detect an association between smoking and TaqI A genotype did not yield any conclusive results.

2.4.6.5 TaqI A polymorphism and alcoholism

Blum et al. (1990) published the first report of an association between alcoholism and the TaqI A1 allele among 35 alcoholics and 35 nonalcoholic controls. Numerous attempts to replicate the finding soon followed. The first meta-analysis three years later found eight case-control studies of TaqI A and alcoholism, with altogether 430 alcoholics and 490 controls, meaning that the number of subjects in each study was generally quite small (Pato et al., 1993). Four of these studies were positive on association, and the other four failed to find any. The authors explained the variance in results by between-study heterogeneity (different forms of alcoholism in different studies) and by within-study heterogeneity (different forms of alcoholism in the same study sample). According to this meta-analysis, as the severity of alcohol dependence decreases, the rate of phenocopies (not genetically determined forms) increases. Also, it was known that the TaqI A1 allele frequency varied significantly between populations, from 9 to 75% (Barr and Kidd, 1993). So the population heterogeneity between the groups of cases and control subjects could significantly mask the real effect or demonstrate a false association. However, there was a statistical support for an association between the A1 allele and alcoholism. When the results of all these studies were put together, the calculated RR was 2.14 (Pato et al., 1993).

The comprehensive review of the association between TaqI A and alcoholism by Noble (1998a) covered 15 US and European studies among white subjects. Over 1000 alcoholics (from severe to less severe forms) and 900 controls (including subjects assessed or not assessed for alcoholism) were involved. The study samples still were quite small, with a lot of heterogeneity between them. The prevalence of the A1 allele varied from 25 to 65% among alcoholics and from 12 to 35% among controls. The homozygous A1/A1 genotype was very rare, about 3% in both groups. Taking only those studies where the severity of alcoholism was defined, the author found a statistically significant difference in the prevalence of the A1 allele between the severe and less severe forms (47.7%, n=285 vs 31.6%, n=351, including genotypes A1/A1 and

A1/A2). Also, there was a significant difference in the prevalence of the A1 allele between control groups not assessed for substance abuse and those from which alcoholics and drug abusers were excluded (31.1%, n=399 vs 15.7%, n=236).

As the findings of the review by Noble (1998a) were taken together, the more severe alcoholic subjects had an A1 allele prevalence three times higher that that of the assessed non-dependent controls. This difference was statistically highly significant. Even the less severe alcoholics had an A1 allele prevalence twice as high as that of the assessed "clean" controls. However, the A1 allele prevalence was almost identical among the less severe alcoholics and the non-assessed controls. The author emphasized two critical factors: the type of alcoholics, and the nature of the controls. Noble (1998a) claimed that population stratification bias was excluded since the analysis consisted of a large number of white subjects from different geographic regions. He concluded that DRD2 TaqI A polymorphism is an important gene in alcoholism, and also in other substance use disorders (Noble, 1998a; Noble, 1998b).

Later studies on TaqI A and alcoholism not listed in the reviews above have also shown confounding results. Hietala et al. (1997) in Finland found a significantly higher A1 allele frequency among 70 alcoholics compared with 50 controls, but no association with the severity of alcoholism. Edenberg et al. (1998b) studied a large family-based sample, genotyping almost 1000 mainly white individuals from 100 families, a sample collected in the COGA study. The TDT and the Affected Family-Based Controls tests were applied, but failed to find either linkage or association between the DRD2 gene locus or TaqI A polymorphism and alcoholism. Konishi and colleagues first studied 130 Mexican American alcoholic men and controls for several polymorphisms, but failed to find any association between TaqI A and alcoholism. Later they expanded the alcoholic study group up to 200, and found an association between both the TaqI A1 allele and TaqI B1 allele and very early onset alcoholism, indicating a severe form of alcoholism (Konishi et al., 2004). In a study among 140 white PTSD (post traumatic stress disorder) patients and controls, the A1 allele

frequency was significantly higher in the heavy drinking half of the patients (Young et al., 2002). In this modest sample of 40 heavy drinking patients, the A1 allele was associated with alcohol abuse, but only if the subject also had PTSD (anxiety symptoms). Berggren et al. (2006) studied 357 male alcoholic inpatients and 800 controls in Sweden, and found an excess of the A1 allele among alcoholics. However, the OR for alcoholism with the A1 allele in their study was low, only 1.34.

2.5 Summary of the literature review

Alcohol abuse and dependence seem to be among the most strongly familial and inherited psychiatric disorders. The lifetime prevalence of these disorders combined is probably as high as 20%. Also, there is a frequent comorbidity with other psychiatric disorders and dependence of other drugs of abuse. Alcoholism is also a heterogenous condition, different forms of this brain disease having different courses. So the model of inheritance must be complex, different subtypes probably having different genetic aetiologies, and the relationship between genetic and environmental impact varying depending on the subtype. Some forms of the disorder may be more strongly genetically determined than others. The observed common comorbidity of alcoholism with other psychiatric disorders increases the heterogeneity, and also suggests the polygenic inheritance of alcoholism. Each of the suspicious alleles adds a piece to the burden of vulnerability, increasing the probability of one or more disorders being expressed.

To obtain reliable results, the study subjects affected with alcoholism should be classified into subtypes modelled on the basis of research evidence. Intermediate phenotypes, more homogenous clinical subgroups with common neurobiology, and in some cases common genetic vulnerability, have been developed for molecular genetics studies to increase the power of association studies. Cloninger's neurogenetic model developed on even earlier research evidence offers a simple way to classify alcoholics into subgroups (type 1 and 2) in clinical interview sessions.

The effects of acute alcohol intoxication on neurotransmission in the animal and human brain indicate involvement of several neurotransmitters. Dopamine and serotonin still appear to be among the most crucial ones. So far, most of the studies of an association between alcoholism and candidate genes have been about genes involved in neurotransmission. The genes involved in serotonin and dopamine neurotransmission have been extensively studied, but the findings have been equivocal. The importance of biological support for the study hypothesis has been emphasized: the use of functional polymorphisms with known effects on brain protein synthesis may yield results easier to interpret.

Concerning the polymorphisms listed above, which are suspected to predispose to alcohol use disorders, it should be noted that only a few are functional mutations. The functional exceptions include alcohol metabolic/pharmacokinetic polymorphisms ADH1B (His47Arg, allele frequencies 0.65/0.35, Southeast Asia) and ALDH2 (Glu487Lys, 0.65/0.35, Southeast Asia), histaminergic HNMT (Thr105Ile, 0.90/0.10), and possibly 5-HT3B encoding for a serotonergic receptor. Also, dopaminergic COMT (Val158Met, 0.51/0.49) and serotonergic 5-HTTLPR (L/S, 0.60/0.40), the crucial polymorphisms in our studies, are distinctively functional, but at the same time very common, as can be seen from the observed allele frequencies (Bevilacqua and Goldman, 2009). On the other hand, there is considerable research interest and activity in GABA receptor genetics, but no functional mutations have been detected yet. Along with new data, this situation can, of course, change very rapidly in the near future.

Alcohol consumption habits in human populations form a continuum, starting from total abstinence and continuing through social drinking, heavy drinking, and abuse to the endpoint of severe dependence and disability. Considering the polygenic nature of vulnerability to alcoholism, it can be suggested that the genetic factors influencing the vulnerability might be expressed, to some extent, among the socially drinking population as well, related to the amounts of alcohol consumed. This indicates a need for studies of suspicious candidate gene

effects among social drinkers, as well, if population samples large enough are available. The ultimate purpose of candidate gene studies, of course, is to find out the differences in the neurobiology of the different forms of alcoholism. This would help in the development and targeting of medical treatments for this disorder.

3 AIMS OF THE STUDY

This study investigated whether there are associations between alcohol consumption in humans and the polymorphisms of selected candidate genes. Alcoholic subjects were classified as type 1 or type 2, as Cloninger (1987; 1995) has suggested. COMT and 5-HTTLPR genotypes were compared between these groups and healthy controls (and the general population) in studies I–III. COMT and DRD2 TaqI A genotypes were studied among a large sample of non-alcoholic socially drinking males in studies IV and V.

The specific aims:

1. To study the functional polymorphism (Val158Met) in the COMT gene (encoding cerebral dopamine inactivation) among alcoholics and controls. I–II

2. To study the functional polymorphism 5-HTTLPR (L/S) in the serotonin transporter gene (coding transporter synthesis) among alcoholics and controls. III

3. To study the functional COMT (Val158Met) polymorphism in a large representative sample of socially drinking males. IV

4. To study the DRD2 gene TaqI A polymorphism (A1/A2) (possibly affecting DRD2 receptor availability in the human brain) in a large representative sample of socially drinking males. V

4 MATERIAL AND METHODS

4.1 Study subjects and diagnostics

The study protocols were approved by the Research Ethics Committees of Kuopio University/Kuopio University Hospital and Turku University/Turku University Hospital (studies I–III), or the Research Ethics Committee of Kuopio University/Kuopio University Hospital (studies IV–V). All participants gave written informed consent to participate in the studies. The diagnoses of the index subjects were made according to DSM-III-R or DSM-IV (American Psychiatric Association, 1987, or 1994). All index and control subjects were white males of Finnish origin. The exact numbers of the index subjects from the same samples in studies I–III or IV–V show a slight variation between the studies, depending on whether the particular genotype studied was obtained in the genotype analysis.

4.1.1 Type 1 alcoholic subjects

The type 1 alcoholic population (total n=123 in studies I–II, and n=114 in study III) consisted of two independent Finnish samples of male alcoholics from the regions of Turku (n=67–65) and Kuopio (n=56–49). The sample in Turku consisted of alcoholics entering a detoxification program in that area, and patients in Kuopio were recruited with the help of a local rehabilitation center for alcoholics, where they had obtained treatment for their alcoholism. The mean age (±SD) of these subjects was 44.1±8.8 years (studies I–II) or 43.8±8.8 (study III). Inclusion criteria were serious alcohol-related problems (alcohol abuse or dependence resulting in a failure to fulfil obligations at work or in recurrent social problems) starting after the age of 25 years. The onset of alcohol-related problems was determined by interviewing the subjects.

All these type 1 patients fulfilled the Diagnostic and Statistical Manual of Mental Disorders' (third edition, revised, DSM-III-R; American Psychiatric Association, 1987) criteria for alcohol abuse or dependence, and underwent a clinical examination and a self-administered Michigan Alcoholism Screening

Test (MAST, Selzer, 1971). Exclusion criteria were major mental disorders such as schizophrenia, schizophreniform and schizoaffective disorders, mood disorders with psychotic features, organic mental syndromes and disorders, and paranoid and other psychoses (screened with the Hopkins Symptoms Checklist 90 and a clinical interview by a physician). Subjects with a history of violence or severe antisocial behavior, or severe physical illness, were also excluded. The crucial differential diagnostic factors for the alcoholic subject to be included in the type 1 alcoholic group were the onset of drinking problems after the age of 25 years and the lack of a violent antisocial history, according to Cloninger's model.

4.1.2 Type 2 alcoholic subjects

The type 2 alcoholics (n=62 in study II, and n=51 in study III) were committed for forensic psychiatric examination in a state mental hospital (Niuvanniemi Hospital, Kuopio) after committing an impulsive violent offense (homicide, attempted homicide, aggravated violent assault, assault, sexual offense, or arson). In Finland, most persons charged with serious violent offenses are committed for forensic psychiatric evaluation: 70% of all homicide offenders, for example, are evaluated (Eronen et al., 1996), and most of the offenders who are considered very violent or dangerous are evaluated in Niuvanniemi Hospital regardless of their residence. Therefore, the offenders included in this study were representative of habitually violent offenders in the Finnish male population. Based on the data gathered, all were recidivist offenders, although just under 10% of them had not been convicted in court before the evaluation. Forty percent of the subjects had committed at least one homicide or an attempted homicide and aggravated violent assaults, and 25% had committed at least 2 homicides or attempted homicides. The mean age (±SD) of these subjects was 30.4± 8.2 years in study II and 30.1±8.4 years in study III.

All type 2 alcoholics were subjected to an extensive forensic psychiatric examination including a psychiatric evaluation, standardized psychological tests, evaluation of physical condition with laboratory tests,

electroencephalography (EEG), magnetic resonance imaging (MRI), and staff observation in a security ward for 4–8 weeks. Inclusion criteria were serious alcohol-related problems before the age of 25 years and the co-morbid diagnosis of ASP disorder fulfilling DSM-IV (American Psychiatric Association, 1994) criteria. Abuse or dependence had resulted in recurrent social problems and recurrent substance-related legal problems, fulfilling DSM-III-R criteria for alcohol abuse or dependence (American Psychiatric Association, 1987). The onset of alcohol-related problems was determined within a 1-year accuracy on the basis of data gathered from various sources during the forensic psychiatric evaluation. Exclusion criteria were major mental disorders such as schizophrenia, schizophreniform, and schizoaffective disorders, mood disorders with psychotic features, organic mental syndromes and disorders, and paranoid and other psychoses or severe physical illness.

4.1.3 Controls (studies I–III)

Previously published data on COMT genotypes of 3140 blood donors (Syvänen et al., 1997) were used as a model of the Finnish general population. There were also 267 unrelated controls (mean age±SD=54.6±6.92 years) from the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) (Salonen, 1988) reporting low or moderate alcohol use (self-reported avarage alcohol intake = 1–7 drinks/week and MCV<101 FI, GGT<80 u/l). (For more details about the KIHD subjects, see below.) The controls in study III were 54 unrelated healthy males from the Turku and Kuopio regions in Finland (mean age±SD=44.1±7.9 years).

4.1.4. The representative sample of socially drinking males (studies IV–V)

The Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) is a population-based epidemiological study launched in the 1980s to investigate previously unestablished risk factors for myocardial infarction, progression of atherosclerosis, and other major health outcomes in middle-aged men (Salonen, 1988; Lakka et al., 1994; Lynch et al., 1994). The total sample of the

KIHD study consists of 2,682 men who were recruited in two cohorts. The present study is based on the second cohort, an age-stratified sample of 42-, 48-, 54-, and 60-year-old men (n=1516, participation rate 82.6%) enrolled in the study between 1984 and 1989. Men who had reported no use of alcohol for at least 12 months were defined as abstainers (n=123, 11% in study IV; and n=100, 10% in study V). Since abstainers are a fairly heterogenous group consisting of lifetime non-drinkers as well as those who have quit because of health problems or other reasons, they were excluded from final analyses. The proportion of abstainers in this sample is similar to the 10% oserved in the general population in Finland (Nylander et al., 2007). From the remaining subjects, both sociodemographic and genotype data (COMT in study IV and DRD2 in study V) were available for 896 subjects and 884 subjects, respectively.

The study population was a random sample of men living in the city of Kuopio, or neighboring rural areas. The mean age (±SD) of the volunteers was 51.9 (±6.73) years in study IV, and 56.1 (±6.7) years in study V. A variety of sociodemographic, behavioral and medical assessments were done according to the KIHD protocol as described earlier (Lakka et al., 1994; Lynch et al., 1994). Age, place of residence (urban/rural), marital status, educational level (represented by a 7-point scale from less than elementary=1 to academic degree=7), current income, history of smoking in cigarette-years, and history of physician-diagnosed chronic diseases (ischemic heart disease, diabetes, stroke, cancer, diseases of the liver or pancreas, mental disorder) and severe trauma were recorded in the questionnaire and double-checked by the research staff in the interview.

A self-report quantity-frequency questionnaire derived from the Scandinavian Drinking Survey (Hauge and Irgens-Jensen, 1981) was used to record the level of alcohol use. The average weekly consumption of alcohol in pure ethanol (g/week) was calculated based on the known alcoholic content of each beverage type and the reported doses and frequencies of drinking sessions (Kauhanen et al., 1997). Serum gamma-glutamyltranspeptidase (GGT) and

mean corpuscular volume (MCV) was determined from baseline blood samples as biomarkers of excessive alcohol use. These biochemical measures were checked to validate the self-report data. The correlation of self-reported alcohol consumption with the GGT and MCV measures separately at various levels of alcohol consumption has been examined earlier. There was no indication of differential misclassification due to erroneous reporting (Kauhanen et al., 1992).

4.2 Genotype analysis

4.2.1 COMT

COMT genotypes were determined by two different methods. In studies I–II genotypes were determined by restriction fragment length polymorphism (RFLP) analysis from DNA extracted from the subjects' peripheral blood by an investigator unaware of phenotype, as described earlier in Lachman et al. (1996). Briefly, the polymorphism is generated by the presence of a G or an A encoding a valine or methionine at codon 158. A 210 base pair 32P-radiolabeled PCR product was generated using the primers 5'-CTCATCAC-CATCGAGATCAA and 5'-GATGACCCTGGTGATAGTGG (nucleotides 1881–1900 and 2071–2090 in GenBank accession number z26491) (Tenhunen et al., 1994). The PCR product (10 μl) was treated with 5 units of Nla III for 3 hr at 37°C and then separated by electrophoresis using 8% nondenaturing polyacrylamide gels (Lachman et al., 1996). The allele sizes for the COMT polymorphism are 85 nucleotides for the H allele and 67 for the L allele. There are two other bands that are common to both genotypes - 54 and 71 nucleotides.

In study IV DNA was extracted from 10 ml of ethylenediaminetetraacetic acidanticoagulated venous blood using standard salting-out or phenol-chloroform assays. COMT genotypes were determined by restriction fragment length polymorphism (RFLP) analysis from the DNA by an investigator unaware of the phenotype. The polymorphism is generated by the presence of a G or A encoding a valine or methionine at codon 158 and recognized by the heat-

shock protein (hsp) 92 I1 restriction enzyme in the presence of A. A 179 base pair polymerase chain reaction (PCR) product was generated using the primers forward 5'-CTGCTGGAGCTGGGGGCCTAC-3' and reverse 5'-AGGTCTTCAG-GAATGC-3'. The reverse primer introduces a one-nucleotide mismatch to remove hsp 92 II restriction site at codon 164. The PCR product (5 μl) was treated with two units of hsp 92 II for 16 hr at 37°C and then analyzed by 2% agarose gel electrophoresis. The diagnostic bands are 179 (valine) and 139 (methionine).

4.2.2 5-HTTLPR

DNA was extracted from venous blood by an investigator unaware of the phenotype. The 5-HTT promoter polymorphism was detected by PCR using the primers TGAATGCCAGCAGCAGCACCTAACCC and TTCTGGTGCCACCT-AGACGC. The PCR reaction was carried out in a 20-μl volume containing approximately 100 ng of genomic DNA and Deep Vent polymerase (New England Biolabs, Beverly, MA, USA). After an initial denaturation step of 96°C for 2 min, the cycling parameters were 40 cycles consisting of 96°C for 30 s, 61°C for 1 min, and 71°C for 1 min. The PCR fragment was radio labelled by including 5 mCi32 PdCTP in the reaction mix. In order to facilitate complete melting of this GC-rich region of the genome, 7-deazaguaine (New England Biolabs) to a final concentration of 0.5 mM was added. The PCR product is a 406/450 base pair fragment that was resolved by electrophoresis through a 4% non-denaturing acrylamide gel and visualized by autoradiography.

4.2.3 DRD2 TaqI A

DNA was extracted from whole blood using standard procedures. Subjects were genotyped by an investigator unaware of the phenotype for the TaqIA RFLP polymorphism of the D2 receptor gene, as previously described (Grandy et al., 1993). The polymorphism was detected by PCR using primers 5'-CCGTCGACGGCTG-GCCAAGTTGTCTA-3' and 5'-CCGTCGACCCTTCCT-GAGTGTCATCA-3'. PCR was performed according to standard conditions of

94°C 1 min, 50°C 1 min, and 92°C 1.5 min consisting of 35 cycles. Digestion of 10μl of the PCR products was accomplished overnight with 5 units of TaqI enzyme under oil at 65°C. Three fragments of 310 bp, 180 bp, and 130 bp show the A1/A2 genotype. The A2/A2 genotype is indicated by two fragments of 180 bp and 130 bp, and the A1/A1 genotype is revealed by the uncleaved 310 bp fragment. All three fragments are resolved on 1% agarose gel and can be visualized by staining with ethidium bromide (Grandy et al., 1993).

4.3 Statistical analysis

4.3.1 Studies I–III

Genotype and allele distributions were analysed and compared using the chi-square test. Odds ratios and 95% confidence intervals were calculated as described by Morris and Gardner (1998). The effect of age was studied (association was adjusted for age) using logistic regression analysis. In study I the population aetiological (attributable) fraction was calculated as described in Armitage and Berry (1994).

4.3.2 Studies IV–V

We compared the age-adjusted weekly alcohol consumption level in the three genotypes using the analysis of variance, checking whether the homogeneity of variance meets the assumptions for the model. Socio-demographic background variables, smoking and prior diseases, i.e. the confounding factors originally selected for the KIHD study in the 1980s (Lakka et al., 1994; Lynch et al., 1994), were controlled for in the multivariate model. SPSS statistical software was used in all analyses.

5 RESULTS

5.1 COMT polymorphism among alcoholics and controls (I–II)

The COMT genotype and allele (L=met, H=val) frequencies are shown in Table 1. The COMT genotypic distributions were in Hardy–Weinberg equilibrium among all the groups of cases and controls. The L allele frequency was higher among the type 1 alcoholic subjects both in Turku (x^2=5.21, P=0.023) and in Kuopio (x^2=8.03, P=0.005) when compared with the general population. When the pooled data from all type 1 cases (n=67+56=123) were compared with the data from the general population (blood donors), the difference was statistically highly significant (x^2=12.7, P=0.0004). The L allele frequency was significantly higher among type 1 alcoholics (pooled together) when compared with matched controls (x^2=6.78, P=0.009), as well. When the genotypes of all type 1 alcoholics (n=123) were compared with the genotypes of matched controls, the odds ratio (OR) for alcoholism for those subjects having the LL genotype vs those with the HH genotype was 2.51 (95% CI 1.22–5.19, P=0.006). The age-adjustment in the logistic regression analysis had only a small effect on the odds ratio (OR for LL vs HH genotype 2.79, 95% CI 1.23–6.35, P=0.015). The odds ratio (OR) for type 1 alcoholism for LL genotype vs LH or HH genotype combined was 1.55 (95% CI 0.95–2.53, x^2=3.43, P=0.064) when compared with controls, and this OR was 1.64 when type 1 alcoholics were compared with the general population.

Table 1. COMT Genotype and allele frequencies

	Genotype			Allele	
TYPE 1 CASES (n=123)	**LL**	**LH**	**HH**	**L**	**H**
Turku (n=67)	22 (0.33)	35 (0.52)	10 (0.15)	0.59	0.41
Kuopio (n=56)	20 (0.36)	30 (0.54)	6 (0.11)	0.63	0.38
Total (n=123)	42 (0.34)	65 (0.53)	16 (0.13)	0.61	0.39
TYPE 2 CASES (n=62)	14 (0.22)	32 (0.52)	16 (0.26)	0.48	0.52
CONTROLS					
Blood donors (n=3140)[1]	0.24	0.50	0.26	0.49	0.51
Healthy subjects (n=267)	67 (0.25)	136 (0.51)	64 (0.24)	0.51	0.49

[1]Data obtained from the study by Syvänen et al. (1997)

Since the controls were from the same area as the alcoholics (type 1) in the Kuopio sample, the allele and genotype frequencies were also compared between these two (more homogenous) populations from Eastern Finland (Kuopio type 1 alcoholics vs matched controls). The L allele frequency was higher among these alcoholics (x^2=5.29, P=0.021) than controls, and the OR for LL vs HH genotype was 3.18 (95% CI 1.11–9.53, x^2=5.83, P=0.016). The population aetiological (attributable) fraction for the LL genotype in alcoholism was 13.3% (95% CI 2.3–25.7%). This was calculated by using the OR obtained for LL vs LH plus HH genotypes in the comparison of alcoholics and the general population (OR 1.64). The respective estimate based on the OR from the comparison with the matched controls from the Kuopio area was 12%. The population attributable risk was calculated from $\lambda=\theta e\ (\phi-1)$: $[1+\theta e\ (\phi-1)]$, where θe is the proportion of the population exposed, and ϕ is the relative risk (= OR).

The type 2 alcoholics did not differ from the general population or controls when compared for allele frequencies or genotypic distribution. Adjusting for age using logistic regression analysis had no significant effect on the similarity of the genotypic distribution of type 2 alcoholics and controls. However, the L allele frequency was significantly higher among type 1 alcoholics (x^2=4.98, P=0.026) when compared with type 2 cases. The LL/LH genotype was more common in type 1 alcoholism than in type 2 alcoholism, the OR being 2.33 (95% CI 1.00–5.41, P=0.030). The OR for type 1 alcoholism when compared with type 2 cases for those subjects with the LL genotype versus the HH genotype was even higher (OR=3.00, 95% CI 1.09–8.37, P=0.017).

5.2 5-HTTLPR polymorphism among alcoholics and controls (III)

The 5-HTTLPR genotype and allele frequencies are shown in Table 2. The genotype distributions were in Hardy–Weinberg equilibrium among type 2 alcoholics and controls, but not among type 1 alcoholics (x^2=11.9, 1 d.f., P<0.001). The S allele frequency was higher among type 2 alcoholics when compared with healthy controls (x^2=8.24, P=0.004) and type 1 alcoholics (x^2=4.86, P=0.028). Since genotype distribution among type 1 alcoholics was

not in Hardy–Weinberg equilibrium, the difference in allele frequency between type 2 and type 1 alcoholics was also studied with the more conservative Cochran–Armitage trend test (P=0.055). The OR for being a type 2 alcoholic compared with controls, and having the SS genotype, as opposed to the LL genotype, was 3.90, 95% CI 1.37–11.11, P=0.011. Adjustment for age in the logistic regression analysis only slightly attenuated the relationship (OR 3.59). For homozygous SS genotype vs LS and LL genotypes, the OR was 3.14, 95% CI 1.12–9.02, P=0.015. There were no statistically significant differences in genotype or allele distributions when type 1 alcoholics were compared with healthy controls.

Table 2. 5-HTTLPR genotypes and allele frequencies

	Genotype			Allele	
	LL	**LS**	**SS**	**L**	**S**
Type 1 (n=114)	50 (0.44)	37 (0.32)	27 (0.24)	0.60	0.40
Type 2 (n=51)	15 (0.30)	18 (0.35)	18 (0.35)	0.47	0.53
Healthy controls (n=54)	26 (0.48)	20 (0.37)	8 (0.15)	0.67	0.33

The table shows the serotonin transporter promoter genotype and allele frequencies among type 1 and type 2 alcoholic subjects and healthy controls (L = Long, S = short allele of 44-bp insertion-deletion polymorphism).

5.3 COMT polymorphism among socially drinking males (IV)

The mean age of the 896 subjects was 51.9 years (SD 6.73), ranging from 42 to 61 years. The COMT genotype and allele frequencies are shown in Table 3. The genotypic distribution in the study population was in Hardy-Weinberg equilibrium (x2=1.15, 1 df, P=0.28). Table 4 shows the distribution of age and other background factors in the three COMT genotype groups. There were no marked differences in any of these variables. Also, there were no significant differences in the biomarkers of alcohol abuse between the three genotypes. The means and standard deviations of GGT were 27.8 units per litre (SD 30.2) in the LL group, 28.1 units per litre (SD 24.8) in the LH group, and 32.8 units per litre (SD 36.9) in the HH group (F=1.82, 2 df, P=0.17). For MCV, the means and standard deviations were 91.9 fl (SD 4.52) for the LL genotype, 92.0 fl (SD 4.28)

for LH, and 92.1 fl (SD 4.77) for HH (F=0.08, 2 df, P=0.93). The Pearson correlation coefficient between alcohol consumption and GGT was 0.27 (P<0.001) in the total sample, 0.24 (P<0.001) in the LL genotype, 0.36 (P<0.001) in the LH genotype, and 0.23 (P< 0.01) in the HH genotype. For alcohol consumption and MCV, the correlations in the total sample and in the LL, LH, and HH genotypes were 0.22 (P<0.001), 0.25 (P<0.001), 0.18 (P<0.001), and 0.23 (P<0.01), respectively.

The age-adjusted weekly ethanol consumption was higher in the LL genotype group (103.4 g/week, SD 168.6) than among heterozygotes (78.7 g/week, SD 192.3) or HH homozygotes (83.1 g/week, SD 123.0) (F=3.09, 2 df, P<0.05). A test for homogeneity of variances (P=0.647) indicated the appropriateness of the analysis. The alcohol use in the LL group was 27% higher compared with the two other groups combined. A multivariate adjustment for age, place of residence, education, income, marital status, smoking in cigarette-pack-years, and history of ischemic heart disease, stroke, cancer, diabetes, liver disease, mental disorder, and severe trauma did not affect the relationship, and the differences in weekly drinking remained statistically significant (F=3.48, 2 df, P=0.031) (Table 5). Of the covariates, young age (P<0.01) and living in an urban area (P<0.05) were associated with higher level of drinking. Smoking showed the expected relationship with a high drinking level (P<0.001). None of the other variables in the model had any association with alcohol use.

Table 3. COMT Genotypes and Allele Frequencies in the Population Sample of 896 Middle-Aged Finnish Men

Genotype (Total 896)			Allele	
LL	**LH**	**HH**	**L**	**H**
269 (30.0%)	428 (47.8%)	199 (22.2%)	0.54	0.46

Table 4. Means (Standard Deviations) and proportions of background variables in the three COMT genotype groups

	LL (n=269)	**LH (n=428)**	**HH (n=199)**	**Total (n=896)**
Age (yr)	52.6 (SD7.0)	51.6 (SD 6.5)	51.7 (SD 6.8)	51.9 (SD6.7)
Living in rural area (%)	24	21	24	22
Annual income (Finnish marks)	87,496 (SD 54,052)	91,504 (SD 56,034)	90,130 (SD 49,833)	90,000 (SD 54,265)
Educational level (1=low, 7=high)	2.01 (SD 1.74)	2.06 (S.D 1.78)	2.10 (SD 1.75)	2.05 (SD 1.77)
Married (%)	88	88	91	89
Cigarette smoking (pack-years)	168.7 (SD 310.1)	170.5 (SD 322.1)	166.0(SD 300.3)	168.0 (SD 312.7)
Ischemic heart disease (%)	24	21	22	22
Diabetes (%)	4	4	2	3
History of cancer (%)	1	2	3	2
History of stroke (%)	1	2	3	2
Liver disease (%)	1	1	2	1
History of mental disorder (%)	4	5	5	5
History of serious trauma ('YO)	7	12	10	10

Table 5. Mean weekly alcohol consumption in pure ethanol among the COMT genotype groups

	COMT genotypes			
	LL	**LH**	**HH**	***p* value**
Age-adjusted mean alcohol consumption (g/week)	103.4	78.7	83.1	0.046
Mean alcohol consumption (g/week) adjusted for all covariates	103.7	77.7	83.8	0.031

5.4 TaqI A polymorphism among socially drinking males (V)

The TaqI A genotypes and the self-reported ethanol consumption (g/week) among the non-abstainer subjects (n=884) are shown in Table 6. The table also shows the weekly ethanol consumption adjusted for 13 covariates. The genotypic distribution of the study population was in Hardy-Weinberg

equilibrium. There was no significant difference in the overall genotype distribution between the 100 abstainers and the 884 non-abstainers (A1A1 2% vs 7%; A1A2 30% vs 35%; A2A2 68% vs 59%). Among the 884 non-abstainers, the alcohol consumption in the homozygous A1A1 group was about 30% lower than in the A1A2 group, and 40% lower than in the A2A2 group (F=3.180, d.f. 2, P=0.042; F=3.200, d.f. 2, P=0.041 in an adjusted multivariate model).

Table 6. Self-reported ethanol consumption (g/week, mean±SD) among the 884 nonabstainer subjects, classified into three groups according to their DRD2 TaqI A genotype

	A1A1 n=58 (0.07)	**A1A2** n=306 (0.35)	**A2A2** n=520 (0.59)	*p* value
Ethanol consumption (g/week±SD)	58±64	82±126	97±133	0.042
Ethanol consumption adjusted for 13 covariates	55	86	97	0.041

6 DISCUSSION

6.1 COMT (I–II)

The most prominent finding was the significant difference between type 1 alcoholics and controls or the general population, and on the other hand, between type 1 and type 2 alcoholics, considering the frequencies of the COMT L (Met) allele or LL genotype. In this regard, type 2 alcoholics were more or less similar to the controls. This would mean that either the effect of dopaminergic COMT polymorphism is insignificant in the development of type 2 alcoholism, or the effect of this polymorphism is different in these two types of alcoholism. On the other hand, previous studies of neurobiology have shown predominantly serotonergic defects in type 2 alcoholism.

A later study using a large sample drawn from the Australian general population (862 white subjects) found no association between COMT genotype and present symptoms of alcoholism (Henderson et al., 2000). Nakamura et al. (2001) could not detect any significant differences in COMT allele frequencies or genotypes between 91 male Japanese alcoholics and 114 controls, not even between the subgroups considering the age of onset of alcoholism or history of violent behaviour. Wang et al. (2001) found a preferential transmission of the L allele to early-onset alcoholic subjects by applying the transmission disequilibrium test (TDT) among 70 German parents/offspring trios. Horowitz et al. (2000) found a significant excess of the H (Val) allele in a group of 38 heroin-addicted Israelis from 35 families compared with 38 controls, using family-based haplotype relative risk testing. However, they failed to replicate their finding when using a case control design among a different but larger group of ethnically heterogenous heroin addicts. Enoch et al. (2006a) studied ethnically homogenous Plains American Indians (342 females and males), and detected a significant excess of COMT H allele among alcoholic subjects.

The studies above, published after our two studies on COMT polymorphism and alcoholism, did not unanimously support or contradict our findings. It is still safe to refer to our original hypothesis made at the end of 1990s, suggesting

that ethanol induces longer-lasting and more effective dopamine release and euphoria when the inactivation rate of this catecholamine is markedly reduced, as is the case with LL carriers. That would favour the development of late-onset type 1 alcoholism. In early-onset type 2 alcoholism with antisocial and impulsive violent behaviour, this would probably have a minor impact. In other words, type 2 disorder is more or less determined by serotonergic deficits starting from an early age, substance use disorders being only one (secondary) symptom of the syndrome.

Since our studies were published, new data have emerged concerning the role of COMT polymorphism and especially showing that L (Met) allele carriers gain better prefrontal cognitive function and also increased vulnerability to anxiety and lower pain threshold. H (Val) allele carriers show the opposite traits. So there is a balance of advantages in better cognitive function vs better stress resiliency - i.e. the worrier/warrior model (Oroszi and Goldman, 2004; Goldman et al., 2005a). As a paradox, the worrier/warrior model would actually explain why H allele carriers might also be vulnerable to the development of substance use disorders (and alcoholism) through impulsivity, being less capable of thorough consideration and planning before acting. The findings by Caspi et al. (2008) supported this showing an association between the COMT H/H genotype and antisocial behaviour in children with ADHD, a combination known to strongly predispose to adult criminality and substance use disorders. Consequently, both of the COMT Val158Met alleles may increase vulnerability to alcoholism through different effects on brain function (Ducci and Goldman, 2008). On the other hand, there was no excess of H allele or HH genotype in our sample of extremely impulsive violent type 2 alcoholics compared with controls: the frequencies were almost identical. Therefore, these results would not indicate any major impact of COMT polymorphism in the development of type 2 alcoholism, at least not in our sample of habitually and extremely violent type 2 alcoholics. These samples of alcoholic subjects, of limited size, indicated only the effect of the COMT L (Met) allele in the predisposition to type 1 alcoholism.

The subgroup of type 1 alcoholics, representing probably the as much as 80% of alcoholics, is not a properly defined intermediate phenotype of alcoholism, being too large and heterogenous. However, the association between the COMT genotype and alcoholism in our sample of type 1 alcoholics was clear. Through clever and careful patient selection, excluding antisocial subjects and those suffering from major mental disorders, we may have found an anxiety-prone subgroup of type 1 alcoholics. After all, patients seeking treatment in detoxification and rehabilitation clinics in Finland, such as the subjects in our sample, do that on their own initiative. This may reflect the fact that they are more inclined to anxiety, and especially feelings of guilt, than their peers. Thus, what we found was that COMT L allele may have increased the risk for the development of alcohol dependence by increasing the vulnerability to stress and anxiety in this subpopulation. They probably had an increased risk for alcohol disorders due to other genetic and environmental factors, as well.

6.2 5-HTTLPR (III)

Our results very clearly showed an excess of S allele among type 2 alcoholics compared with healthy controls or type 1 alcoholics. In this regard, there was no significant difference when type 1 alcoholics were compared with controls. Considering genotypes, the risk was greatest for the homogenous SS group versus LL carriers (OR 3.90). For the SS genotype versus LS and LL genotypes combined, the risk was somewhat lower (OR 3.14). Adjustment for age is crucial, because type 2 subjects according to the definition and to their risk-taking lifestyles tend to be younger, and are at risk of early death, compared with type 1 subjects with a more social life history. However, adjustment only slightly attenuated the difference here, the OR for SS genotype versus LL remaining 3.59.

The published studies since the end of the 1990s have reported contradicting results on associations between 5-HTTLPR and alcoholism. A study involving almost 1000 Japanese alcoholic subjects and matched controls could not find any association in general, but the SS genotype was significantly more common

among binge drinking alcoholics (Matsushita et al., 2001). Kranzler et al. (2002) studied 471 mainly European-American early-onset alcoholic subjects and controls, and Johann et al. (2003) 534 German alcoholics (with comorbid ADHD) and controls. Neither of these studies could detect any association. Parsian and Cloninger (2001) observed an excess of the L allele among 130 white alcoholics compared with controls. A study with 350 Korean male alcoholics and controls also found an association between the L allele and alcoholism (Kweon et al., 2005). There have also been numerous reports of an association between the S allele and alcoholism (total sample sizes including index subjects and controls in parentheses): among French alcoholics with suicidal behaviour (170) (Gorwood et al., 2000); among German and Hungarian alcoholic probands (90) and their parents (Lichtermann et al., 2000); among German and Hungarian alcoholic individuals with suicidal behavior (280) (Preuss et al., 2001); among European-American alcoholic subjects with depression (550) (Nellissery et al., 2003); and among Mexican-American alcohol-dependent subjects (451) (Konishi et al., 2004). A trend towards a similar association was observed in studies by Thompson et al. (2000) and by Stoltenberg et al. (2002).

A comprehensive meta-analysis (Feinn et al., 2005) gathered data investigating associations between 5-HTTLPR alleles and alcoholism from 17 studies involving 3500 alcoholics and 2300 controls, most of them of European ancestry. Fifteen of these studies used a case-control design, and two used the transmission disequilibrium test (TDT). There was an overall trend towards an association between the S allele and alcoholism. Of the potential moderator variables (year of publication, sample size, sex or age, S allele frequencies among alcoholics or controls, non-European descent, diagnostic criteria used, or co-occurring clinical feature), only the co-occurring clinical feature had a significant effect. These included antisocial behavior, early onset, or severe form of dependence, suicidal behaviour, ADHD, depression, or Tourette's syndrome. These co-occuring features explained the majority of the between-study variance in a random-effects model. For all the studies combined, the OR

for the S allele was 1.18, indicating that the S allele increased the odds by at least 18% for an individual to be diagnosed with alcoholism. However, the studies among alcoholics with a co-occurring clinical feature yielded an even higher OR of 1.22.

Based on the data available at the end of the 1990s, 5-HTTLPR polymorphism in this study was genotyped as consisting of two alleles. The later reports of triallelic 5-HTTLPR polymorphism, where L alleles are classified into high-expressing La and low-activity Lg, do not agree on the functional impact of these two L alleles. However, La alleles would still constitute the majority of the L alleles, the frequency of low-acitivity Lg alleles in white populations being 0.09, at most. Even Hu et al. (2006) speculated, after introducing the triallelic 5-HTTLPR, that the main result of ignoring the triallelic nature of this polymorphism and not scoring the low function Lg allele, is to obscure the effect of the highest expressing LaLa genotype. Hu et al. (2006) concluded that the effect would be less crucial for phenotypes previously associated with the low activity S allele. Unfortunately, re-analysing the genotypes was not possible in the present study.

Low-activity SS genotype is suggested to lead to lower production of serotonin transporter (5-HTT), and theoretically to higher levels of serotonin in the synaptic cleft. How would this explain the reduced serotonin transmission observed in type 2 alcoholics? One explanation could be that the excess of synaptic 5-HT is probably present in early childhood already, in individuals who are later in danger of developing the symptoms of type 2 alcoholism. This constant early excess of 5-HT would induce desensitisation of various postsynaptic 5-HT receptors. Alternatively, or simultaneously, an increase in synaptic 5-HT stimulating presynaptic 5-HT autoreceptors would lead to a decrease in presynaptic 5-HT release. Either way, the net effect would be a decrease in the brain 5-HT transmission, clinically expressed as an antisocial, impulsive and violent lifestyle, combined with substance use disorders, including alcoholism.

There is a considerable body of data supporting Cloninger's neurogenetic model. This evidence indicates that childhood antisocial conduct disorder preceding adult-onset antisocial personality disorder with habitual impulsive violent behaviour is actually the core syndrome, with alcoholism and abuse of other substances being secondary, not the cause of the behavioural deviation. Considering this, we may have found an association between an endophenotype and alcoholism. In other words, the association was not between the 5-HTTLPR S allele and alcoholism per se, which would just have added another argument to the contradictory findings on this polymorphism. Instead, we found an association between this transporter polymorphism and an impulsive, habitually violent antisocial behavioural pattern with alcoholism and other substance abuse (in other words, type 2 alcoholism). The antisocial, impulsive, habitually and extremely violent alcoholics carefully screened for this study represent the "hard-core" of the antisocial alcoholics. Samples consisting of only similar index subjects, corresponding to our sample selection, have not been used in other studies of alcoholism. Obviously, there are great difficulties in collecting a sample like ours, as these offenders usually are non-compliant to any medical intervention. This may in part explain why true replications of this study have been uncommon, and our study has remained more or less unique.

6.3 COMT polymorphism among socially drinking males (IV)

In this study we replicated the finding of an association between alcohol consumption and COMT genotype, observed among type 1 alcoholics in studies I and II, among a large number of non-alcoholic socially drinking males in Eastern Finland. The effect of the low-activity COMT L (met) allele in increasing weekly alcohol consumption was not dose-dependent. Both groups of homozygous subjects (LL and HH genotypes) consumed more than heterozygous LH subjects. This does not indicate a very strong single gene effect or dominance of the L allele. Nevertheless, the differences between the LL carriers and the two other genotype groups were clear and significant, the LL group consuming 27% more than the other two groups combined. Even after

controlling for the confounding factors (multivariate adjustment), this difference remained significant.

The obvious conclusion to draw from the results would be that the COMT genotype has an impact on the development of alcohol consumption habits, among other genetic and environmental factors. Our original explanation for this was the same as for the association between COMT and alcoholism that we discovered in our first study: individuals with low activity LL genotype may experience a more intense and slowly fading euphoria induced by the alcohol releasing dopamine in the brain. This still sounds like a logical interpretation of this later finding as well.

On the other hand, as discussed above concerning studies I and II, we have the two-way characteristic of the COMT genotype that probably played a role in keeping both alleles very common in the human genome. This means the balance of gaining better cognitive function but worse stress and pain resiliency for carriers of the L allele, and slightly diminished executive cognitive performance but higher pain threshold and better resilience to stress for H allele carriers. The LL genotype would lead to excessive worrying and vulnerability to alleviating the stress by drinking. Again, the LL group, which consumed most alcohol, may have consisted of worriers, as was speculated in connection with the link between COMT and type 1 alcoholism. On the other hand, the association between the COMT L allele and better cognitive performance is based on the evidence of brain imaging studies in humans (Egan et al., 2001; Oroszi and Goldman, 2004; Goldman et al., 2005a). It was predicted that the COMT genotype would make a difference in available dopamine, measured with metabolic activity of the human frontal cortex during cognitive performance, because the levels of dopamine transporters are low in this brain region. The COMT capacity then becomes rate-limiting in dopamine turnover. From this point of view the hypothesis of longer-lasting dopamine induced euphoria while drinking alcohol is supported, too.

In the results of this study we can also see the effect of the H allele in drinking, though only as a trend, not as a statistically significant finding.

Homozygous HH men consumed more alcohol than heterozygous LH males. This has been found in some studies of alcoholism as well. It has been explained by the dyscontrol or impulsivity of the HH subjects, the warrior-like features (Goldman et al., 2005a). Altogether, the results corroborate the conclusions that Ducci and Goldman (2008) presented in a recent review, concerning the alternative ways through which the COMT polymorphism can affect the development of alcohol use disorders. The background information collected from these subjects, originally recruited for an epidemiological study investigating cardiovascular risk factors, was not precise enough to allow further conclusions about the differences in alcohol consumption between the genotype groups. Again, subtyping into defined intermediate phenotypes might have yielded more information, considering the high number of subjects genotyped.

6.4 TaqI A polymorphism among socially drinking males (V)

We found an evident significant association between TaqI A genotypes and weekly alcohol consumption among this large sample of ethnically homogenous, non-alcoholic middle-aged males. The study design was almost identical to that of study IV, on a different polymorphism, but still with a suspected impact on dopaminergic neurotransmission and reward pathways in the human brain. The earlier research on neurobiology and reward mechanisms, as well as Cloninger's neurogenetic model of alcoholism, strongly suggest an association between decreased availability of DRD2 receptors and markedly increased amounts of consumed alcohol (in alcoholism). Also, most previous studies on TaqI A polymorphism seem to suggest a weak association between the A1 allele and alcoholism, though there have been numerous conflicting findings as well (a meta-analysis by Smith et al., 2008). An existing association would be a reasonable conclusion, because the A1 allele has been claimed to associate with decreased DRD2 availability in humans (Pohjalainen et al., 1998; Jönsson et al., 1999; Hirvonen et al., 2004). However, our results did not corroborate these suggestions: they indicated an opposite association of decreased DRD2 availability and reduced weekly alcohol consumption.

Unfortunately, the functional impact of TaqI A on DRD2 availability has not yet been solved. It was originally suggested that this polymorphism is in a linkage disequilibrium with another more functional polymorphism affecting DRD2 availability. However, there is a serious problem in this notion, since TaqI A does not lie anywhere near the DRD2 gene, and could not possibly regulate the expression of this gene. Recently, TaqI A was located in an adjacent ANKK1 gene, possibly affecting dopaminergic transmission through cellular responses (Neville et al., 2004). It was later shown to be associated with the enzymatic biosynthesis of dopamine (Laakso et al., 2005). There is also a reported linkage disequilibrium between TaqI A and C957T polymorphisms, the latter located in the DRD2 gene with a suggested functional effect on the receptor, possibly even shared with TaqI A. Consequently, it is still possible that the studied TaqI A is functional and related to DRD2, either on its own, by a mechanism so far unknown, or through LD. After the discovery of the connection of TaqI A with the ANKK1 gene, possible family-based associations in 220 white COGA families (n=1923) were re-analysed. The results suggest that there are several genes clustered in the chromosomal region containing the DRD2 gene which may be involved in the risk for alcoholism. The involvement of multiple genes may also provide an explanation for the inconsistency in the literature surrounding the role of DRD2 in alcoholism (Dick and Bierut, 2006).

The latest meta-analysis by Smith et al. (2008) gathered 44 studies, with almost 9400 participants, investigating the association between TaqI A polymorphism and the risk for alcohol dependence. They found a small but significant association in both dominant and recessive modes of gene action (higher risk for A1A2 and A1A1 genotypes, respectively), with an odds ratio of 1.38 (95% CI 1.20–1.58). Given the modest effect size of the meta-analysis, the authors speculated that many of the original studies included may have been underpowered: as more studies were published in the course of time, the pooled odds ratio decreased approaching one. Because of the polarity of opinion on the role of TaqI A, the publication bias may not have markedly hindered the publishing of negative findings, showing a lack of association. The

lack of ethnic matching, as well as inadequate blinding or screening of controls, may explain the heterogeneity observed between studies. The authors concluded that TaqI A remained one of the possible polymorphisms affecting the multigenetic risk for alcoholism (Smith et al., 2008).

Our hypothesis when planning the study among non-alcoholic subjects was originally developed after the report of Phillips et al. (1998) showing low ethanol preference and consumption in knock-out mice totally lacking DRD2 receptors. Also, we were dissatisfied with most of the previous studies on TaqI A, because they generally included very few subjects with the A1A1 genotype. The previous findings in small ethnically varying samples were also hard to generalize because the allele frequencies showed a considerable variation in different populations. In our study we had a large ethnically homogenous and representative population sample of Finnish males. Since they were not alcoholics, we were not looking for an association with alcoholism, and the genetic aetiology of social drinking habits might not be the same as that of alcoholism. Whatever the functional impact of TaqI A is, this impact would not probably be very specific for alcoholism. The aetiologies of these two lifestyles - alcoholic or socially drinking - are probably different, and even the effect of the same genes may differ in them. This last assumption seems reasonable considering the reviews of the subject (the latest by Smith et al., 2008) still showing an association between alcoholism and TaqI A1 allele. This is quite the reverse to our findings among non-alcoholic drinkers.

Our results confirmed our original hypothesis, showing that males with the A1A1 genotype (and presumably the lowest DRD2 density) consumed 40% less alcohol than A2A2 males with the highest DRD2 density, if associated with TaqI A polymorphism. Heterozygous subjects showed an intermediate consumption, indicating a dose-dependent effect of the A1 allele in these non-alcoholic subjects. As shown in Table 6, the standard deviation in drinking quantities was the smallest in the least-drinking A1A1 group, suggesting that they are homogenous concerning their drinking habits. Munafò et al. (2005) also had two large population-based cohorts of white subjects from the UK, originally

randomly selected to participate in a study on genetic associations with tobacco smoking. The final study samples consisted of 400 and 500 subjects of both sexes, aged 35–75 years. Abstainers were excluded, which led to quite high exclusion rates from the original samples (67% and 76%). Also, men consuming more than 50 units per week and women consuming more than 35 units per week were excluded to avoid the inclusion of alcoholic subjects. This last exclusion may still have left some heavy drinkers of both sexes in the study samples. A significant association was found between the TaqI A1 allele and reduced alcohol consumption, the effect being stronger in men (A1/A1 and A1/A2 genotypes combined vs A2/A2 genotype, A1/A1 being rare, n=39). The authors thus replicated our findings, even though in their study the number of subjects after exclusions was considerably smaller than in ours.

The observed association with alcohol consumption is most easily explained by the proposed association between TaqI A genotypes and DRD2 availability. Non-alcoholic individuals with low densities of DRD2 may not gain as much pleasure from drinking and the extra dopamine released, because they lack the postsynaptic binding sites of dopamine which mediate reward and euphoria. This interpretation is not in conflict with that offered in the discussion concerning COMT polymorphism among the same subjects. In that study we had basically intact dopamine release and binding sites, only the effect of dopamine was sustained in subjects with slower inactivation of this transmitter. We are actually talking about the same phenomenon in these two contexts: more euphoria with longer lasting dopamine effect, or less euphoria with subtle dopamine transmission. Considering the development of alcoholism, there very probably are several other genetic and environmental factors with an equal or stronger impact. The TaqI A genotype is just one risk factor among others, acting differently in subjects vulnerable to alcoholism than in non-alcoholic individuals.

7 SUMMARY AND CONCLUSIONS

1. The results of these five studies on the functional dopaminergic and serotonergic polymorphisms showed significant associations in different populations consuming different amounts of alcohol. This further confirms the evidence from previous research indicating crucial roles of dopamine- and serotonin-mediated neurotransmission in the development of alcohol use habits, and alcohol abuse and dependence, i.e. alcoholism.
2. Alcoholism is a heterogenous disorder. Type 1 alcoholism, the category containing the majority of alcoholics, showed an association with the dopaminergic COMT polymorphism, but not with the serotonergic 5-HTTLPR polymorphism.
3. Type 2 alcoholism, with ASP and habitual impulsive violent behaviour, showed an association with the serotonergic 5-HTTLPR polymorphism, but not with the dopaminergic COMT polymorphism.
4. The dichotomy of the results on COMT and 5-HTTLPR polymorphisms among type 1 and type 2 alcoholics is consistent with the dopaminergic and serotonergic deficits suggested previously concerning these subtypes of alcoholism. These subtypes are based on Cloninger's neurogenetic model, which was originally developed in the 1980s, before the era of molecular genetics, but has gained support from later molecular genetics studies, including studies I–III here.
5. The dopaminergic polymorphisms COMT and TaqI A showed an association with alcohol consumption among socially drinking males, indicating the role of dopamine-mediated reward mechanisms in regulating non-alcoholic drinking patterns.
6. Careful diagnostic procedures, classification and subtyping of alcoholic study subjects are essential given the heterogenous nature of this disorder. Comorbid disorders should be screened for to support the formulation of clinical subtypes.

An association study alone does not prove causality. In linkage, haplotype or candidate gene association studies there is always the possibility of missing a true association or finding a false one by chance. The use of complicated statistical analysis in genetics epidemiology has also met criticism, especially multiple testing. However, there was a substantial body of research data on two differentially inherited types of alcoholism with different behavioural traits, and data supporting differences in dopaminergic and serotonergic neurotransmission between the two types of alcoholism. Consequently, the results of studies I–III concerning these subtypes, and the observed dichotomy at the genetic level, were more or less a confirmation of previous observations.

Connected to the COMT studies I–II, study IV also seems to stand on sound reasoning, with quite reasonable results. Only study V lacks a strong theoretical background, because there was no clear evidence of the functional biological impact of the TaqI A polymorphism. New data have emerged, but the possible functional nature of TaqI A has not yet been clarified. However, it was one of the most extensively studied polymorphisms in alcoholism in the 1990s. Our study subjects were not alcoholics, and our results contradict previous results on this polymorphism in alcoholism. However, our results confirmed our original hypothesis, and the role of dopamine and reward mechanisms in regulating drinking patterns.

The obvious recommendation for the future would be careful diagnosing and the subtyping of alcoholic subjects during clinical interview sessions, whenever possible. The screening of comorbid mental disorders, not just major mental disorders, is of utmost importance. This applies not only in research but also in clinical treatment planning. At the moment, there are few effective medical treatments for alcoholism in general. This is particularly the case in antisocial type 2 alcoholism, but also the treatment and prevention of type 1 alcoholism essentially is the treatment of comorbid depression and anxiety. For type 2 subjects at the moment, preventive methods at an early age, even before the childhood conduct disorder is fully developed, are probably the treatment of choice. Given these basic clinical facts, the importance of screening for

comorbid disorders is clear when the purpose is to assess the realistic choices of treatments for an alcoholic patient.

Considering the conflicting results of the previous or later research, we very probably would not have obtained any significant results if we had studied our alcoholic subjects as one group, without subgroups. The sample sizes should have been many times larger than we had in order to discover any association among unclassified alcoholics. The large sample sizes we had in studies IV–V are still uncommon in psychiatric genetics studies, and even here we were studying an epidemiological population sample which was not designed for a study of alcoholism and did not consist of affected individuals. Because collecting large samples of affected individuals in psychiatry is a vast effort, defining subtypes, i.e. intermediate phenotypes, is strongly recommended in future studies. Another recommendation would be to concentrate on functional rather than non-functional polymorphisms when candidate gene association studies are concerned. This strategy will probably yield significant findings with reasonable interpretations. However, genome-wide association studies in the near future may vastly increase the possibilities to reveal the genetic determinants of various diseases and health-related phenomena in general.

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