

Chapter 9.

Summary and Discussion

In this thesis, smoking and co-occurring addictive behaviours were investigated with the help of several genetically informative designs. Below I first summarize the most important results per chapter and then discuss these findings within a broader context.

Summary

In *chapter 3*, a simple question on smoking expectancy ('Do you think you will smoke in a year's time?') predicted future smoking behaviour in never and former smokers, but not in current smokers. This was tested by measuring smoking expectancy and smoking status at baseline, and then assessing smoking status again two years later. These analyses were corrected for a number of confounders among which age, gender, education, self-reported health and in the case of (former) smokers, (former) smoking frequency and quantity. Whether or not an individual predicted their future smoking behaviour correctly was partly heritable. Genetic factors explained 59% of the variation in the ability to predict future smoking in adolescents and 27% in adults. The remainder was explained by unique environmental factors in both adolescents and adults.

The aim of *chapter 4* was to elucidate the mechanism behind spousal resemblance for smoking. First, findings from previous studies were confirmed by showing that smoking behaviour of spouses correlates more than would be expected by chance. An individual who smokes was more likely to have a spouse who smokes as well, and vice versa. For both ever smoking and current smoking, spousal resemblance was higher for a more recent compared to a less recent cohort (cohorts: 1997-2000, 2000-2005 and 2009-2013). This increase was mostly driven by a rise in the number of couples in which neither smoked. A higher age of men was associated with a lower spousal resemblance for ever smoking. By utilizing data of twins and spouses, it was shown that the resemblance between spouses in smoking behaviour was most likely due to phenotypic assortment. Under phenotypic assortment, spouses select each other on phenotype and are therefore genotypically more similar than two randomly paired individuals. Since smoking is moderately to highly heritable this has consequences for the offspring of smoking parents, which will, on average, have a higher genetic risk of smoking.

In *Chapter 5*, observational associations between smoking behaviour and caffeine consumption through coffee, tea, cola and energy drinks were tested in a typical 'coffee-drinking country' (the Netherlands) and a typical 'tea-drinking country' (the United Kingdom). After correction for age, gender, education and social class, we found a positive association between smoking and caffeine use. This association was consistent across the two countries and for total caffeine, coffee and cola. For tea use, there was a negative association in the Dutch sample (smokers consumed less tea) and a positive association in the British sample (smokers consumed more tea). A higher age was associated with a higher consumption of total caffeine, coffee and tea but with a lower consumption of cola and energy drinks. Women consumed less total caffeine, coffee and cola than men. In the Dutch sample women consumed more tea than men while there was no association between gender and tea use in

the British sample. Finally, a higher educational level was associated with a lower consumption of total caffeine, coffee, cola and energy drinks. Again, in the Dutch sample a higher educational level was associated with a higher consumption of tea while there was no association in the British sample.

In *chapter 6*, explanations for the observational association between smoking and caffeine consumption as reported in chapter 5 were explored with three methods: bivariate twin modeling, LD-Score regression and Mendelian randomization analysis. The first two methods were utilized to estimate the correlation between genetic influences on smoking and genetic influences on caffeine consumption, while the third method was employed to explore causal effects. Results were remarkably consistent in showing that there was a considerable genetic correlation between smoking and caffeine consumption ($r_g=0.4-0.5$). The positive observational association between smoking and caffeine consumption was mostly due to these correlated genetic factors. Mendelian randomization analysis provided no evidence for causal effects of smoking on caffeine consumption or of caffeine consumption on smoking, but this may have been due to a lack of power. These findings suggest that the initiation of smoking may be especially undesirable for individuals who use a lot of caffeine. Given their genetic susceptibility they are more likely to also smoke more heavily or to more easily become nicotine-dependent.

Chapter 7 focussed on the heritability of sugar consumption and the association with substance use. Consumption of sugar-containing drinks (e.g. soft drinks, coffee or tea with sugar) was measured, as was the use of five addictive substances (nicotine, alcohol, caffeine, cannabis and illicit drugs). By employing a bivariate twin model, it was tested whether sugar consumption (high vs. low consumption of sugar-containing drinks) and substance use (high vs. low substance use) were associated and whether this association was due to genetic and/or environmental factors. We found that sugar consumption was 48% heritable with the remaining variation being explained by unique environmental factors (52%). For substance use this was 62% and 38%, respectively. There was a moderate genetic correlation between sugar consumption and substance use ($r_g=0.24$). Overall, these findings indicate that sugar consumption is influenced by genetic factors to a considerable degree and that neuronal circuits underlying the development of both addiction and obesity may be related. The unique environmental correlation was $r_e=0.20$, suggesting that there are also environments that influence both sugar consumption and substance use (e.g. social situations).

Finally, *chapter 8* describes a study that puts forward evidence for an adverse effect of smoking on attention problems. Such a causal association had been suggested in animal research, but there was no convincing evidence from human research yet. In this study, the discordant monozygotic (MZ) co-twin design was applied. This genetically informative design tests whether smoking causally leads to more attention problems by comparing the attention problem score of a twin who has smoked with that of his or her co-twin who has never

smoked. Because MZ twins are genetically almost identical and grow up in the same family, the design corrects for confounding of genetic factors and shared family environment. We found that in adult twin pairs discordant for smoking, the smoking twin had significantly more attention problems than their non-smoking cotwin. With longitudinal data it was shown that during adolescence, when neither of the twins smoked, this difference in attention problems did not yet exist. These results provide further support for the hypothesis that smoking causally increases attention problems, as suggested in animal studies.

Discussion

The results of this thesis corroborate with the large body of existing literature in showing that addictive behaviour (including smoking, caffeine use and high sugar consumption) is moderately to highly heritable. Gaps in the literature have also been addressed by focusing on the nature of the associations between different types of addictive behaviour, by studying the mechanisms underlying spousal resemblance for smoking and by exploring the (causal) effects of smoking on attention problems. Here I will discuss the most important findings of this thesis in a broader context and reflect on their possible implications.

Identifying groups at high risk of smoking

Smoking is one of the most harmful addictive behaviours when considering its contribution to morbidity and mortality (1). It is desirable to prevent the initiation of smoking as much as possible, especially since the heritability of nicotine dependence (75%) is much higher than that of smoking initiation (44%) (26). It is becoming increasingly clear that delivering treatment or preventive measures with a personalized approach is more effective than providing one generic program for all (108). In order to personalize preventive efforts in the field of smoking, the identification of risk groups may be useful. When individuals who are at high risk of smoking are identified, preventive measures can be either personalized or targeted so that those who are most vulnerable to smoking receive the highest possible benefit. One way of distinguishing individuals at high risk of smoking from those at lower risk is by enquiring about someone's expectations. Smoking expectancy, which was explored in chapter 3, reflects a single, simple question and is capable of predicting future smoking behaviour in never and former smokers. Measuring smoking expectancy could thus be a reliable and easy way of defining never smokers who are at risk of initiating smoking and former smokers who are at risk of relapsing. Similar single-item measures for identifying risk groups have been investigated in previous studies. Kotz, Brown, & West (2013) investigated the predictive value of the 'Motivation To Stop Scale' (MTSS), a single-item measure with seven answer categories, designed to predict which smokers will attempt to quit smoking in the future and which will not. The MTSS provided a strong and accurate prediction of quit attempts in current smokers (85). In another study, 'susceptibility to smoking' was measured in never-smoking adolescents. This single-item measure aimed to predict which adolescents would start smoking in the future and it was defined as not being able to rule out the idea of smoking one year later (dichotomous variable). Adolescents who were susceptible to smoking were much more likely

to initiate smoking than those who were not (79). A big advantage of smoking expectancy, as presented in this thesis, is that it can be applied to individuals of all smoking statuses. This is in contrast to the two other two single-item measures described here. However, after correction for confounders smoking expectancy was not able to predict future smoking status in current smokers. This poorer predictive value of smoking expectancy in smokers was mostly driven by incorrect expectancies of smokers who said they would ‘certainly not’ or ‘probably not’ smoke in a year’s time, but who did still smoke two years later (see Table S1 in chapter 3). Such incorrect expectancies emphasize how difficult it is for smokers to stop smoking. It has been noted many times that most smokers attempting to quit will fail in remaining abstinent. One study showed that only 3%-5% of self-quitters (those quitting without treatment/help) achieved prolonged abstinence for 6-12 months after a quit attempt (88, 89). An explanation for the greater predictive value of the MTSS in smokers could be that its ability to predict quit attempts was tested, instead of prolonged abstinence as we tested (85). In conclusion, it is demonstrated in this thesis that a single-item measure can be useful when aiming to predict future smoking behaviour. Such information could be of use for prevention programs with the goal of preventing smoking initiation in youth. It may for instance be worthwhile to start off a school-based intervention program by assessing the risk of smoking with a question on smoking expectancy. Those at higher risk can then be given a personalized program, while all others receive a generic intervention.

Apart from asking people about their own views with single-item measures such as smoking expectancy, another indication for being at high risk of smoking can be derived from chapter 4 of this thesis. In that study, spousal resemblance for smoking was explored and it was found that such resemblance was due to phenotypic assortment. Under phenotypic assortment, spouses select each other based on their phenotype which means that the offspring of two smoking parents is at higher genetic risk of smoking (84). The heritability of nicotine dependence (75%) is higher than that of smoking initiation (44%) (26). Thus, the increased risk in children of smoking parents especially relates to their vulnerability to become dependent to nicotine after smoking is initiated. From this it follows that they can benefit most from programs aimed at preventing the initiation of smoking (when they do not start smoking, they cannot develop nicotine dependence). Such preventive programs may increase in effectiveness when the smoking status of parents is employed in order to identify high risk groups. After high risk adolescents have been identified, their personal views or expectations about smoking could also be incorporated. For instance, a child of two smoking parents who scores high on smoking expectancy (thus thinking it is likely that he/she will smoke in a year’s time) would be at the high end of risk for smoking. An approach where prevention is personalized depending on the risk of smoking may be more (cost-)effective than the current method of delivering one, generic prevention program to all school-going youth. This is of particular importance given the disappointing effects of school-based interventions. For example, a Dutch school-based prevention program consisting of lessons on knowledge, attitudes and social influences had a positive effect on high-SES children only (254). Another

study showed that a Dutch school-based prevention program that is applied by ~75% of all secondary schools in the Netherlands was not effective at all (regardless of SES group) (255). These findings stress the need for more effective school-based approaches to prevent smoking. This may be achieved by identifying (high) risk groups and by applying more personalized approaches. Variation in the initiation of smoking is explained by the environment for 56% with most of this estimate consisting of common environment influences (51%). This includes the family environment and thus parents (26). Another advice would therefore be to involve parents (more) in the prevention of smoking. A recent Cochrane review study provided moderate quality evidence that family-based interventions have a positive effect on preventing smoking initiation in children and adolescents (256).

Genetic overlap between addictive behaviours

Results in chapters 6 & 7 demonstrated that the clustering of different addictive behaviours (smoking and caffeine use, substance use and sugar consumption) was for a considerable part due to genetic factors. This has previously also been shown for example for smoking, alcohol and caffeine use (157) and for the association between disordered gambling and smoking, alcohol and caffeine use (257). This thesis and the current literature thus indicate that certain genetic variants increase a person's risk of using several addictive substances and/or engaging in more than one addictive behaviour. Obvious candidates for such genetic variants are those that code for receptors of neurotransmitters that are involved in the brain's reward system, such as dopamine (258) or serotonin (259). Significant associations between genetic variants located in or near dopamine receptor genes or serotonin transporter genes and measures of alcohol use/dependence have been found through candidate gene studies and GWAS (260). For smoking initiation, coffee consumption and BMI, there is also evidence for association with a gene that affects the dopaminergic system. This gene (*BDNF* gene) codes for a neurotrophin that regulates the survival of dopaminergic neurons (33, 164, 206). In addition, a gene that codes for a protein that converts dopamine into norepinephrine (*DBH* gene), was associated with smoking cessation (33). When searching for genetic similarities between substance use and sugar consumption, genetic variants coding for opioid receptors may also be of interest. The opioid receptor gene *OPRM1* was associated with having higher preferences for sweet and fatty foods and measures of overeating and BMI, but also with dependence on alcohol, heroin and cocaine (261-264). Recently, another interesting finding was published. A genetic variant in the *CHRNA5-A3-B4* gene region, robustly associated with the number of cigarettes smoked per day in smokers, predicted an increased BMI and waist and hip circumference in non-smokers (207, 208). Together, the findings described here suggest that there are general genetic factors that influence the (in)ability to resist rewarding stimuli. However, much is still unknown about the exact genes that are involved in the risk of addictive behaviour and it is becoming increasingly clear that the development of both substance dependence and obesity is determined by a complex interplay of numerous environmental and genetic factors (265, 266). A next step would be to further assess which genetic variants are involved in the

development of addictive behaviour, and to what degree these variants overlap between the different kinds of behaviour.

A recent approach to estimating genetic correlations, which was also applied in chapter 6 of this thesis to data on smoking and caffeine use, is LD-Score regression (162, 163). This technique estimates the genetic correlation between two traits by utilizing effect-size estimates of all SNPs that are included in genome-wide association (GWA) meta-analyses. Briefly, the expected product for the Z scores of the association between a SNP and the two phenotypes is modelled as a function of the linkage disequilibrium (LD) the SNP has with all neighboring SNPs (i.e. the LD-score). An interesting application of LD-Score regression would be to test the genetic correlation between substance use and sugar consumption. In chapter 7 of this thesis we found a genetic correlation of 0.24 through bivariate twin modeling. It would be good to complement this analysis with a genetic correlation based on effect-size estimates from GWA meta-analyses. At the moment this is not possible because no GWAS on sugar consumption have been published. As an alternative for sugar consumption, summary statistics of GWAS on BMI could be utilized. As such it would be possible to study the overlap in genetic variants associated with BMI, which is causally increased by high sugar consumption (50), and substance use. A recent overview of LD-Score regression findings included a significant genetic correlation of 0.29 between BMI and cigarettes per day while SNPs for BMI and ever vs. never smoking correlated 0.20 (163). These results emphasize the importance of further research to the aetiology of high sugar consumption and the (genetic) overlap with other addictive behaviours.

For now, the most important conclusion is that individuals who are highly dependent on one substance, such as nicotine, are more likely to also be or become dependent on another, such as caffeine. From this it follows that individuals who are dependent on multiple substances probably have a high genetic susceptibility to addictive behaviour in general, and they may therefore find it more difficult to remain abstinent than others. It may also be that those wanting to quit using one (harmful) substance, could best switch to using another (less harmful) substance as a 'substitute'. This kind of harm reduction has for example been proposed for cannabis as an alternative to alcohol, prescription drugs and/or illicit drugs (267, 268). Under this assumption it would be easier to stop smoking when switching to the use of (large amounts of) caffeine. It is unlikely that this holds true for smoking and caffeine however, given the fact that caffeine consumption has been associated with failed smoking quit attempts and induced craving for cigarettes (43, 269-271). In chapter 7 the consumption of different combinations of substances (including smoking, alcohol, caffeine, cannabis and illicit drugs) were described (Figure S1). In a group of men and women who used two substances, the most common combination was smoking-alcohol, closely followed by alcohol-caffeine and smoking-caffeine. For those using three substances the most frequently occurring combination was smoking-alcohol-caffeine. A few studies explored associations between these often co-occurring substances in clinical samples and in some cases explored the relationship with

treatment outcomes. Men with both nicotine dependence and alcohol dependence were found to have higher levels of the Nicotine Dependence Syndrome Scale (NDSS) than men with nicotine dependence only (272). In a group of alcohol-dependent men and women, those who were current smokers and nicotine-dependent individuals had a greater severity of alcohol dependence than those who did not smoke/were not nicotine dependent (273). A final study measured caffeine consumption and family history of alcoholism in pregnant women, and tested the women's ability to reduce caffeine consumption during pregnancy. Interestingly, caffeine-dependent women with a family history of alcoholism were not able to reduce or eliminate caffeine use during pregnancy while caffeine-dependent women without a family history of alcoholism were able to do so (274). It is important that health professionals working in (clinical) practice are aware of such associations and the possibly underlying (genetic) mechanisms.

Causal effects of smoking

In chapters 6 & 8 the causal effects of smoking were explored. In chapter 8, the effect of smoking on attention problems was tested with the powerful discordant MZ co-twin design. The results pointed to a causal increase of attention problems due to smoking. It is the first time that such causality was indicated in human data and it emphasizes that smoking can have detrimental effects not only on physical, but also on mental health. As discussed in a commentary on our findings by London (2015), previous studies have provided evidence that there are differences between smokers and nonsmokers on many executive functioning domains, including attention problems but also cognitive impulsivity, working memory and risk taking during decision making (275). Future studies are needed to test whether these differences are also the result of smoking. The most obvious implication of these findings is that smoking initiation should be prevented or at least delayed as much as possible. One way of achieving this is by increasing the legal age at which someone is allowed to smoke or buy cigarettes. In the Netherlands, the legal age at which cigarettes (and alcohol) can be bought has been raised from 16 years to 18 years in 2014 (245), but our results imply that this may not be enough. Smoking twins still differed from their non-smoking co-twin if smoking was initiated at 18 years or older, implying that it is still detrimental for the developing brain at that age. An example of a stricter and possibly more suitable policy is that implemented in the city of New York, where a law raising the minimum age to smoke to 21 years was adopted in 2013 (247).

More studies are necessary to strengthen the evidence for a causal effect of smoking on attention problems, and thereby further assess the need of increasing the legal age of smoking. To obtain stronger causal inference from observational data, multiple (genetically) informative study designs can be and need to be applied (276). Besides the discordant MZ co-twin design, another way of testing causal effects of smoking is through Mendelian randomization analysis (MR). This technique employs genetic variants as a proxy, or an instrument for a particular trait, which reduces effects of confounding and reverse causation

(156). Future research could include MR analysis to test the effect of smoking on attention problems/ADHD symptoms. MR is increasingly being used to study presumed causal effects of smoking, among which the possible adverse effects of smoking on mental health (277). One example is the nature of the association between smoking and depressive and anxiety disorders. So far, research findings in this area were inconsistent. Some suggested that smoking causally leads to depression/anxiety (278) or the other way around that depression increases smoking (self-medication hypothesis) (279), while others concluded that the association arose from shared familial factors (230). When MR analysis was carried out in >120,000 individuals, there was no evidence for a causal effect of smoking heaviness on depression or anxiety (280). Another large MR study of >63,000 individuals also provided no evidence for a causal influence of smoking on depression, while a direct effect of smoking on psychotic conditions (e.g. schizophrenia) seemed likely (281). It would be suitable to perform similar MR analyses in order to test the causal effect of smoking on attention problems.

When reflecting on the causal effect that smoking may have on attention problems, an important group to consider is that of early adolescents who are diagnosed with ADHD and/or those who suffer from attention problems. In previous, longitudinal work it has been shown that youth diagnosed with ADHD are more likely to initiate regular smoking (56). This may be because these individuals are more impulsive and therefore more prone to experiment with cigarettes, or because they use cigarettes as a type of self-medication. In this thesis it has now been shown that the direction of causality can also go from smoking to attention problems. For adolescents who experience attention problems even before smoking is initiated, this effect of smoking may be most disadvantageous. It therefore seems justified to put more effort into preventing smoking in adolescents with ADHD/attention problems. Informing adolescents with ADHD better about the possible risks of smoking for attention problems might deter them from initiating smoking. In a qualitative study, 39 children and adolescents diagnosed with ADHD (aged 9-17 years) were interviewed about their experiences in everyday life related to the disorder. All participants described that they struggled with their symptoms and reported problems related to school and school achievements (282). Given the problems that youth with ADHD/attention problems themselves report, they may be more open to warnings about (relatively) short-term effects of smoking on attention problems, than they are to warnings about long-term risks such as lung cancer and cardiovascular disease.

In chapter 6, Mendelian Randomization was applied to study the association between smoking and caffeine use, and particularly to test if smoking causally influences caffeine use, or vice versa. No evidence for causal effects was found. As discussed in chapter 6, this may have been due to low power. When assuming that there are no causal effects, it would not be necessary to, for example, adjust caffeine consumption when trying to quit smoking. There is no consensus about the (causal) nature of the association between smoking and caffeine yet, however, since other studies did find evidence for causality. Some experimental and animal studies have suggested that smoking causally increases caffeine use (150-152) while others

reported that caffeine use causally increases smoking (153-155). In contrast, a recent study found a causal effect such that caffeine decreases the number cigarettes smoked per day (181). Overall, the evidence is inconclusive and more and larger (MR) studies are needed to figure out the causality in this relationship. Even though we did not find direct causal effects, there was a considerable overlap in the unique environmental influences on smoking and the unique environmental influences on caffeine use. These findings imply that some environments can evoke both the urge to smoke and the urge to consume caffeine. This information may be important when trying to quit smoking. Environments where one would normally consume caffeine are likely the same environments where one would normally smoke and may therefore best be avoided in the first stages of a quit attempt, when the risk of relapse is the highest (88, 89). When caffeine consumption and smoking of cigarettes often occur at the same time, this could evoke an indirect reciprocal interaction where the use of one substance acts as a cue to use the second substance (43). This line of reasoning is supported by research showing for example that having a coffee in a café or at home after lunch/dinner induced craving for cigarettes in adult current smokers (270, 271). It may be that this is only important for adults, because adolescent smokers who were measured 3 weeks after a quit attempt did not show a lower self-efficacy to stay quit after having consumed coffee (283).

Future research into novel addictive behaviours

The prevalence of smoking has steadily decreased over the past years. In 1991-1997, 65.8% of men and 56.8% of women had ever smoked, while 38.0% and 33.2%, respectively were current smokers. By 2009-2013 this had decreased to 47.7% and 41.8%, respectively for ever smoking and 15.6% and 15.3%, respectively for current smoking. Smoking prevalences in the NTR were somewhat lower than in the general Dutch population in 2014 where 60% of men and 50% of women had ever smoked, while 28% and 22%, respectively were current smokers (2). This slight bias is most likely due to a relatively high proportion of highly educated participants (107), for which we corrected throughout this thesis by including education as a covariate. Along with the decrease in smoking of regular cigarettes, there is currently a rise of 'novel' addictive behaviours such as the use of e-cigarettes and water pipe (also referred to as 'hookah' or 'shisha'). In future studies, it is therefore likely that the focus will shift more towards such traits. The debate on the pros and cons of e-cigarettes is still ongoing, with the biggest concerns being their potential health effects and the possibility that non-smokers will start using them (284-286). As for water pipe, users tend to underestimate, or are not aware of, the negative health effects (287, 288). In an analysis of data from the 2011-2014 National Youth Tobacco Surveys in the US, it was found that while the use of cigarettes is on the decline this is accompanied by increases in the use of e-cigarettes and water pipe. As a result, there was no change in overall use of tobacco-containing products, in spite of the decrease in cigarette smoking (289). In this thesis I present evidence for a causal effect of smoking on attention problems. Animal research has suggested that this causal effect works through nicotine that is inhaled through cigarette smoke (8). This would mean that while the use of e-

cigarettes and/or water pipe may be less detrimental when it comes to the long-term risks of developing cancer or cardiovascular disease, both may still have a detrimental effect on attention problems. This also emphasizes the need to better understand the aetiology of the use of products such as e-cigarettes and water pipe. As a first step it would be interesting to explore the heritability of such behaviours. Another important question to ask is whether the (genetic and environmental) risk factors for using e-cigarettes and water pipe are the same as the risk factors for using regular cigarettes. For a decisive answer on such questions more (twin) studies are necessary.

Another emerging and interesting area of research is the 'addictive' potential of particular nutrients such as sugar or of unhealthy foods. I looked at the heritability of sugar consumption and its overlap with substance use and found that sugar consumption was partly heritable (48%) and that there was a moderate genetic correlation with substance use. There is no scientific consensus yet about whether a particular nutrient such as sugar or other foods can be considered addictive (45, 189, 204, 205, 290). Although 'food addiction' is a relatively new topic, the addictive potential of other, non-substance related, behaviours such as gambling and gaming or internet use have been investigated for some time. In participants of the NTR it was shown that compulsive internet use in adolescents was for 48% genetic in nature (291), while the heritability of pathological gambling was 50%-60% in American twins (292). The aetiology of the consumption of sugar and unhealthy foods and the role that environmental and genetic influences play, are becoming more and more important in today's society. A high consumption of sugar/unhealthy foods contributes greatly to the increase in overweight and obesity (49, 50). Therefore, the influence of genetic and environmental factors on such behaviours needs to be studied and it should be explored which genetic variants underlie the heritability for these traits and whether or not these are genes that are common to multiple addictive behaviours.