Relationship between oral cleft malformation and maternal drinking during pregnan...

Background and Introduction

The problem that has been examined in this study is the relationship of oral cleft malformation in Norwegian infants, maternal drinking during pregnancy, and the infants' and mothers' ADH1C gene variants.

Boyles, DeRoo, Lie, Taylor, Jugessur, Murray, and Wilcox (2010) conducted a population-based case-control study that examined the risk of oral cleft in offspring of mothers who consumed alcohol during the first semester of pregnancy, depending on differential alcohol metabolism of mothers and their fetuses. The authors aimed to find correlation of alcohol dehydrogenase 1C (ADH1C) variants with consequences of maternal drinking during early pregnancy. They hypothesized that if the mothers and fetuses had fast alcohol metabolizing ADH1C variant, the fetuses would be less severely affected by maternal alcohol intake, since they would be exposed to the alcohol for shorter period of time than fetuses of mothers who, both fetus and mother, had a slow metabolizing ADH1C variant. The authors did not specify how they planned to explore the relationship of ADH1C variants with oral cleft in offspring. Their hypothesis was that the two variants of ADH1C differentially alter deleterious effects of maternal alcohol intake. The authors did not specify the variables.

The literature review is very short, and is uniformly supporting the study aim. There is no discussion about many possible gene alterations associated with oral clefts that can be found in literature. This might be useful, since the eligible families needed only to live in Norway at the time of the child's birth, and the mothers needed to speak Norwegian, which did not imply their

ethnicity or the country of origin. From this reason, choosing one gene that is fitting the Norwegian population seems insufficient. The authors intended to study the metabolic variants of ADH1C of both mothers and fetuses, claiming that both had influence on the length of fetal alcohol exposure. The clefts can occur until two and half months since conception. According to Niimi (2008), fetal alcohol dehydrogenase (ADH) starts forming from the two and half months from conception, which is the time after the clefts could be formed. Then the ADH levels rise slowly, therefore the fetus is practically unable to metabolize the alcohol until the birth. Therefore, the claim that the fetal ADH1C variant could influence the rate of alcohol metabolism and the length of fetal exposure to alcohol in utero seems ungrounded. The study authors also did not consider other factors contributing to the rate of alcohol metabolism in pregnant women. For example, according to Niimi (2008), maternal estrogens slow down the alcohol metabolism in correlation with their blood levels produced by the mother. The estrogens increase compared to nonpregnant woman 10-100 times during the first semester of pregnancy. Since this increase has so wide variability, so will vary the maternal metabolism of alcohol.

Methods

The researchers conducted a population-based case-control study of alive infants born in Norway between 1996 and 2001 with oral clefts. They cited their other study that should describe the recruitment in detail, but that described the same as this study. They tried to enroll all identified infants with oral clefts, and the participation was high (88% for cases, 76% for controls). It was not clear, if the cases were chosen from all identified for

surgery, or those that actually came to the two centres for corrective surgeries. The authors did not specify how many dead infants with oral clefts and how many mothers not speaking Norwegian they excluded from the study. The authors acquired 573 cases (systematic sampling) and 763 controls (random sampling), not matched by demographics, age, or sex of infant. The 1: 1. 3 ratio of cases and controls is not ideal for reaching high statistical power. The ratio closer to 1: 3 or 1: 4 would be ideal. The statistical power was not addressed. The frequency of the disease in population could be calculated to 0. 2%, which is low.

During data collection, errors could be introduced if the subjects provided else's than own DNA samples, for example from fear of revealed paternity issues. In this study, this possibility might be relevant, because the paternal DNA was examined in order to exclude from the study infants with Mendelian inconsistencies. If the infant's DNA could not be explained from the maternal or paternal DNA combination, this infant would be excluded from the study due to the Mendelian inconsistence. The researchers collected two different types of DNA samples in cases and controls, but since the DNA is the same from any body sample, this did not influence the results. Mothers filled questionnaires. This mode of data collection could introduce many errors, such as recall bias (family recall bias) about family history of oral cleft – when cases might be better informed about the oral cleft occurrence in their families than were controls, recall bias or insufficient knowledge about pregnancy characteristics, and most importantly, a recall bias and possibly prevarication bias about the alcohol consumption during pregnancy. The mothers were at 14-15 weeks postpartum probably very tired, which could

markedly affect their recall about the events during their pregnancy. The data collection time was about 13-10 months from conception and the end of the first trimester, which seems a long time period to ask persons to recollect their average drinking habits. The study describes questioning mothers about their average weekly or monthly drinking. Given that the oral clefts were forming between 5 and 10 weeks of pregnancy, the timing of the alcohol drinking seems crucial. This problematic is similar to timing of Fetal alcohol syndrome (FAS), whose facial markers occur after maternal alcohol consumption in 19-21 days post conception, and therefore the mother's alcohol intake at any other time has other deleterious effects on the fetus, but not facial characteristics of FAS (Johnson, 2011). In case of oral clefts, maternal alcohol intake before and after the critical cleft closing period is irrelevant for this study. Other literature states that the amount of alcohol the mothers consumed in the critical time period was associated with the probability and severity of oral clefts (Dievert, 1985). Collection of average consumption per week and month could not provide such information, although the authors asked about the amount consumed per sitting. Again, the possibility of recollection of amounts of alcohol used per sitting during the critical period might be low. The amount of drinks consumed clumped into 1-4 and over 5 could cause information being missed. The authors did not explain why they used this way of categorization of their data. The Centre for Disease Control and Prevention defines binge drinking from 4 drinks up (CDC; www. cdc. gov/alcohol). The study authors provide some information from their results already in data collection section (association of the amount of alcohol used with oral clefts). The study authors did not attach the copy of their questionnaire and did not describe how or when they

obtained information about potential confounders. They described how many subjects did not return acceptable material, but in different section than collection of data

In their sample processing section, the researchers don't describe the gene and SNP selection, the data control and their processing. They refer to other publication where they should be described. They did not mention name or type of DNA analysis assay, described a bioinformatics software for analysis of genetic data. The authors used multivariable analysis for which had large enough sample size (for population size 300000 and confidence interval 95% needed 384 subjects; (http://www. raosoft. com/samplesize. html).

The authors claim that ADH starts forming in placenta already in first semester. Their reference (number 12) search, found claim that ADH is present in the placenta in first semester, which could mean anytime until week 13 of the pregnancy. Their claim was based on its two references, from which only one mentioned ADH formation in the placenta, based on their research of fetuses old 9-22 weeks, clumped in one sample without differentiating the age further. The search of the literature through UAA Consortium library and on PubMed did not reveal other mention of ADH expression timing in first trimester. Research through Google scholar revealed one paper/essay describing the ADH presence in the liver of the fetus from 2. 5 months of pregnancy, with minimal ability to influence the alcohol breakdown (Niimi, 2008). Given that the ADH presence and its amount being sufficient to influence the alcohol metabolism in the fetus in first semester, and particularly during weeks 5-10, is the basis for this study hypothesis, the literature that was used to back this claim seems insufficient

or contradictory. The study authors could be investigating a gene that was not available to the fetus during the studied period. This would have implications for subject assignment. The researchers formed pairs of mothers and their infants, based on the ADH1C variants in both. It might make more sense to omit the infants in this study. Maternal ADH1C haplotypes could influence the speed of alcohol breakdown. The authors differentiated between a " high-activity" homozygous subject pairs, and " reduced activity" heterozygous (intermediately metabolizing) and homozygous (slowly metabolizing) subject pairs based on their ADH1C variants. They did not explain if the variants could be dominant and recessive to each other, when it such case, if fast variant were dominant, one would be enough to observe fast metabolism. If it were recessive, its effect could not manifest itself. The assignment of infants might be irrelevant. Clustering of mothers in a particular way might be unfounded.

The study authors checked for Hardy-Weinberg equilibrium. This does not seem be relevant for this study (Andrews, 2010). More, the study authors were probably not checking a homogenous population to make inferences about its DNA changes, since even if they called it a " Norwegian" population, the criteria for subject inclusion did not include having Norwegian ancestors. The researchers again introduced results of their study before the Results section (e. g., an association of oral cleft type with maternal alcohol intake). The assignment according to Michaelis-Menten enzymatic kinetics: The fast variant (homozygous) has a turnover of 90. minute, KM of ethanol is 1. 0mM. The slow variant (homozygous) has a

turnover of 40/minute, KM of ethanol 0. 6mM. The turnover means how many molecules of alcohol per minute get converted to acetaldehyde.

According to Niimi (2008), the metabolic rate of alcohol is 7g per hour. This speed is reduced in pregnant mothers, increasing time of metabolizing alcohol by 1. 5 times. This number is variable because of variable amount of estrogents present in mother's body, which are slowing down the effect of ADH. According to Niimi, the amount of estrogens raises 10-100 times in first semester compared to nonpregnant women. Therefore, the speed of alcohol breakdown can hardly be deduced only from the type of ADH haplotype. Given that some subjects had mixed haplotypes, their unknown dominance/recessivity, and unknown levels of estrogens in blood of mothers at the time of oral cleft formation, the assignment of subjects to groups might be irrelevant even if the infants were omitted.

An alternative study design that might better serve to describe an association of alcohol intake and oral cleft formation could be a prospective cohort study, following up with mothers from pre-conception to ensure measurements in critical window of cleft formation, measuring blood alcohol levels or other means of frequent follow-up measurement of maternal alcohol intake.

Results

The authors explained how they controlled for confounders, which seem to agree with other literature. The authors describe a " striking pattern" of their results, which in some cases seem contradictory to expectation, such as

heavy drinking of mothers acting as a protective factor against oral clefts in offspring.

The results were not statistically significant. The confidence interval has large interval. The P values are large numbers for maternal haplotypes. Small chi square numbers describe that the data the researchers observed should fit with what they expected to find in their study.

There seemed to be quite many adjustments to the data in this study. It might be a disadvantage of availability of powerful statistical analyses, allowing for combinations of available data, to see what combinations would yield most plausible results. There was not a clear line from the introduction, through the methods and analysis, which data exactly would be used to test the hypothesis. There were exclusions of subjects with a strong alcohol metabolizing ADH1B, data were unadjusted and adjusted, groups were subdivided, and calculations were made outside of previous statements about analyses, but results were not shown, only briefly described lated in discussion. This was important because for example consideration of mothers alone changed the results of the study, making claims of ADH1C causality in maternal alcohol intake irrelevant.

The misclassification could influence the study results in several ways: for example by choice of alcohol intake categories, assigning groups according to gene variants, or considering mother DNA together with infant DNA.

Discussion

The authors listed multiple possible issues with their study. Most of them agree with possible issues pointed earlier in this critique, such as results obtained by chance, possibility of recall bias in mothers since alcohol drinking by pregnant mothers is a socially undesirable activity, the unexpected results that don't correspond to dose-response model. The researchers admitted examining data for mothers only, but did not provide results, only description of " unclear association" of alcohol drinking and oral clefts. The study authors claimed that their study supported causal relationship of alcohol consumption and oral cleft formation, and that their data are coherent. This claims seem contradictory to their findings, since their data showed a protective role of alcohol for high-activity ADH1C, with binge drinking more protective than moderate drinking in adjusted sample. Further, the risk of oral cleft is around three times higher in intermediate activity binge drinking group than in low activity binge drinking group. This contradicts other literature and also researchers' own claims that the amount of alcohol consumed in one sitting is more important than frequency of drinking.

Practically, the study results have minimal effect for the disease prevention or treatment. The population will not be examined for gene variant so it could adjust its drinking habits to prevent oral clefts. The authors base their credibility for the research on their results about a fast-variant protecting against oral clefts in binge drinking. The rest of the data does not fit expectation. The gene itself is associated with oral clefts, which makes it confusing to make deductions if it is good or bad to have this gene and its variations, and what caused oral clefts, if the gene or the alcohol, or the

gene-alcohol interaction. They discuss results of analyses for which they did not show data.

Conclusions

Different study design might yield more accurate data on maternal alcohol consumption. An interaction of genes with the environmental factors should be accounted for, as many studies suggest (e. g., Xu et al., 2018) . A cohort study could be designed, including pregnant women in their) early pregnancy. This might mean recruiting subjects who planned to get pregnant, to be able to follow them up already in fifth week of their pregnancy. The first results would be available in ten months, although the study might take as long as the desirable amount of cases would be collected.

If the study results were correct, they might not bring a lot of information about the causality, as they claimed to bring. In the introduction, the authors wanted to add evidence about alcohol causation in oral cleft formation. The knowledge about alcohol involvement in oral cleft formation was documented in other literature. The information about ADH1C gene in the matter seems rather confusing, since the authors claim that the gene should also be associated with the alcohol drinking, which suggests a multiway relationship of alcohol, gene, and risk of oral clefts. More, the role of alcohol in oral cleft formation applies only in a narrow window of opportunity, between weeks 5 and 10, and has to be used in certain amount for the effect. Many other risk factors are associated with oral clefts. The researchers believe that the large sample and the large amount of

information they obtained. They concluded that women should be more cautious in drinking during pregnancy. They hope to use their data in identification of other deleterious effects of alcohol on empbryonic and fetal development, as well as effects on childhood development.

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