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Oral Dis. Author manuscript; available in PMC 2011 January 1.

Published in final edited form as:
Oral Dis. 2010 January ; 16(1): 11–19. doi:10.1111/j.1601-0825.2009.01587.x.

Folic Acid and Orofacial Clefts: A Review of the Evidence

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Abstract

Orofacial clefts are common and burdensome birth defects with a complex genetic and environmental etiology. The contribution of nutritional factors and supplements to the etiology of orofacial clefts has long been theorized and studied. Multiple studies have evaluated the role of folic acid in the occurrence and recurrence of orofacial clefts, using observational and non-randomized interventional designs. While preventive effects of folic acid on orofacial clefts are commonly reported, the evidence remains generally inconsistent. This paper reviews the findings of the main studies of the effects of folic acid on orofacial clefts, summarize study limitations, and discuss research needs with a focus on studying the effects of high dosage folic acid on the recurrence of oral clefts using a randomized clinical trial design. The role of folic acid in the prevention of neural tube defects is also briefly summarized and discussed as a reference model for orofacial clefts.

Keywords

Orofacial clefts; cleft lip; folic acid; folate; prevention; randomized clinical trials

Folic acid is a vitamin that has been shown to prevent the occurrence and recurrence of neural tube defects (NTDs) but findings for its effects on other common birth defects including orofacial clefts (OFC) remain generally inconsistent. Multiple studies of various designs (primarily observational case-control) have evaluated the effects of folic acid and multivitamin use on OFC with an overall suggestive evidence for a potential preventive role of folic acid, but the evidence remains largely inconclusive. In this paper, we summarize the results of the main previous studies of the effects of folic acid on OFC and also briefly summarize the results for NTDs. We also discuss the needs for future research in this area.

Orofacial Clefts (OFC)

Orofacial clefts (OFC) of the lip and palate are common birth defects of complex genetic and environmental etiology. Depending on geographic ancestry, OFC affect about 1 in 500 (Asian or Amerindian ancestry) to 2,500 births (ancestry) (Mossey and Little, 2002). OFC are one of the most prevalent birth defects in the United States, with about 20,400 cases born between 1999 and 2001 (CDC, 2006). Low socioeconomic status is also reported to increase the risk of OFC (Murray et al., 1997; Clark et al., 2003; Durning et al., 2007).

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OFC is thought to result from a complex interplay of genetic and environmental factors. In humans, a finely choreographed cascade of gene expression, cell migration, cell transformation and apoptosis between 14 and 60 days post conception creates the soft and hard tissues of the face from the originating oropharyngeal membrane. By 48 days the upper lip is continuous and by 60 days palatal shelf fusion completes facial embryogenesis (Sperber, 2002). Disruption of any of the tightly regulated processes occurring in this time frame by environmental and/or genetic abnormalities may then predispose to cleft lip and/or palate. A few specific genetic contributors to cleft etiology have begun to be identified including variants in IRF6 (Zucchero et al., 2004, Rahimov et al., 2008, MSX1 (Lidral et al., 1997, 1998; Jezewski et al., 2003), FGF signaling pathway genes (Riley et al., 2007), BMP4 (Suzuki et al., 2009) and a locus on 8q (Birnbaum et al, 2009) but the majority remain unexplained (see reviews in Lidral and Moreno, 2005 and Jugessur and Murray, 2005). Gene-environment interactions also contribute to OFC with strong evidence for interaction between maternal smoking fetal variants of GSTM1 and GSTT1 (Lammer et al., 2005; Shi et al., 2007).

OFC include cleft lip with or without the palate (CL/P) as well as palate only (CP). CL/P and CP are sometimes differentiated in studies due to differences in embryologic origin and recurrence risks, but they are also combined in many studies due to common genetic and epidemiologic risks (van den Boogaard et al., 2000; Dode et al., 2003; Jezewski et al., 2003; Zucchero et al., 2004; Jugessur and Murray, 2005). Recently the role for subphenotypes in clefts has also provided new insights into etiologies (Rogers et al, 2008; Suzuki et al, 2009). OFC occur in both isolated and non-isolated forms. Isolated or nonsyndromic forms involve no other major structural or developmental impairments and represent the majority of cases with CL/P (Jones, 1988; Marazita, 2002). The non-isolated or syndromic forms with CL/P occur due to more than 450 causes including chromosomal anomalies, single gene conditions, environmental exposures, and syndromes of unknown cause (OMIM, 2009). OFC impose significant health, psychosocial, and economic burdens, both at the individual and family levels (Berk and Marazita, 2002).

Folic Acid and Neural Tube Defects (NTDs)

There is strong evidence from clinical trials for a large preventive effect of folic acid on both recurrence and occurrence of NTDs. The strongest evidence for a preventive effect of high dose folic acid supplementation on recurrence of NTDs comes from the Medical Research Council (MRC) double-blinded randomized study, where women with a previous child with NTD were randomly assigned to groups of 4 mg folic acid, vitamins other than folic acid, vitamins with 4 mg folic acid, and placebo, taken daily at preconception and throughout the first trimester of pregnancy (MRC, 1991). The study reported a significant reduction of about 72% in the rate of NTDs in the groups supplemented with folic acid compared to the other study groups. No significant decreases in NTD recurrence were observed in the group receiving vitamins without folic acid, indicating that preventive effects were due the folic acid component (MRC, 1991).

Multivitamin supplementation with a 0.8 mg folic acid at preconception and through at least two months post conception was also shown to lower the risk of first occurrence of NTDs by up to 100% in a randomized clinical trial in Hungary using a sample of women with no history of NTDs among their children (Czeizel and Dudas, 1992). This same study showed no decrease in the occurrence of OFC though the overall rate of congenital anomalies was reported to have decreased with the multivitamin supplementation (Czeizel et al, 1992). In a confirmatory study applying a two-cohort controlled design in Hungary with the interventional group receiving the same folic acid containing multivitamin as Czeizel and

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Dudas (1992) study, Czeizel et al. (2004) found a significant decrease in NTD occurrence by up to 89% and in cardiovascular defects (40%), but no decrease in OFC.

Berry et al. (1999) reported that the use of 0.4 mg folic acid before conception and in the first trimester of pregnancy decreased the occurrence of NTDs in China by up to 79% in a sample from the northern area with higher baseline rates of NTDs compared to 16% in a sample from the Southern region sample with lower baseline rates. Several observational studies have also identified preventive effects of folic acid on NTDs [see a recent review by Wolff et al., (2009)].

The results of the studies described above strongly indicate that the preventive effects on recurrence and occurrence of NTDs are due to the folic acid component rather than the other vitamins, though interactive effects have not been thoroughly evaluated. The NTD research provides a model for developing clinical trials aimed at assessing preventive effects of folic acid on recurrence and occurrence of OFC, which is of direct relevance for clinical practice. A connection between NTDs and OFC can be supported by their similar time of occurrence during embryogenesis, their status as defects involving the midline of the embryo, their near identical population genetic characteristics (variable by geographic origin but with near identical recurrence risks and very similar birth prevalence rates overall), evidence of similar gene and environment contributions and the failure to identify major genetic factors for either.

The mechanisms by which folic acid might prevent NTDs or other birth defects remain unexplained. It might be secondary to the need to overcome pharmacogenetic deficiencies in women who require higher baseline intakes to reach therapeutic levels. One proposed mechanism relates to antibodies to the folic acid receptor (Rothenberg, 2004; Cabrera et al., 2008). The role of antibodies to the folate receptor has yet to be confirmed but could explain why some women respond to high doses of folic acid as this may be required to titer the effects of antibody bound to receptors. The pharmacologic rescue by high dose folic acid has been reported in a rat model where folate receptor antibodies induced intracellular folate deficiency associated with birth defects (da Costa, 2003).

Folic acid and OFC

The role of vitamins and especially folic has been of special interest in OFC for over 20 years. We summarize below the main studies and designs that evaluated the role of folic acid in OFC.

Observational Studies of Folic Acid and OFC

Some observational studies have reported a preventive effect of folic acid containing supplements (mostly multivitamins) on OFC (Botto et al., 2004). However, the evidence is mixed, likely due to sample selection biases as well as differences in samples sizes/statistical power, populations, analytical models (including accounting for confounders), and folic acid measures. Several studies analyzed small samples that may have been underpowered to detect any potential significant effects of folic acid use on OFC. However, these studies have provided important insights into the potential preventive effects of folic acid on OFC. Below, we review the main observational studies in this area.

Using data from the Hungarian Congenital Anomaly Registry, Czeizel (2004) reported that use of high doses of folic acid (average of 6 mg) in the first month of pregnancy reduced CP risk by 50% but not CL/P risk. Shaw et al. (1995) reported a 50% decrease in CL/P with using folic acid containing multivitamins in samples from California but found a smaller and insignificant effect for CP. Van Rooij et al. (2004) reported A similar decrease in CL/P risk

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with using folic acid supplements (mostly containing only folic acid) in a sample from the Netherlands. This study also reported a 74% reduction in CL/P risk with using the folic acid supplements in addition to a high folate diet (Van Rooij et al., 2004). Wilcox et al., (2007) reported a 39% decrease in CL/P risk with using folic acid supplements adjusting for the use of multivitamins and a 64% decrease when women used multivitamin/folic supplements with high folate diets. No preventive effects were observed for CP (Wilcox et al., 2007). Another recent case-control study of a smaller sample of affected births with OFC and controls (compared to the previous study) from Scotland and England found no effects of supplement and dietary folate on OFC (including CL/P and CP; Little et al., 2008). Some studies found suggestive yet statistical insignificant effects of folic acid on OFC (Bille et al., 2007), but others found no effects of folic acid (Shaw et al., 2006). Other studies of multivitamin use without specification of folic acid content have also reported a reduction in risks of CP (by 60%; Werler et al, 1999), CP and CL/P (by 40%; Loffredo et al, 2001), and CL/P (50%; Itikala et al, 2001). One observational study (Hayes et al., 1996) has reported an increased but statistically insignificant risk for CL/P with folic acid containing supplements, yet their control group included children with birth defects (other than midline defects), which might reflect a potential severity reduction effect of folic acid for those anomalies.

With the relatively frequent number and mixed evidence of observational studies, meta-analyses of these studies may be helpful for estimating the average effects of folic acid across several studies and samples. In a metal-analysis of the most recent observational studies, Johnson and Little (2008) estimated a reduction of about 18% in the risk of CL/P with the use of folic acid containing supplements, but no significant reduction in CP. This meta-analysis also found a reduction of about 23% in CL/P risk with using multivitamins (Johnson and Little, 2008). However, it is impossible to identify the effect of folic acid from the effect of multivitamin use in these studies given that most multivitamins may have contained folic acid. In an earlier meta-analysis, Badovinac et al. (2007) estimated a reduction of about 28 and 20% in the risks of CL/P and CP respectively with using folic acid containing supplements and/or multivitamins.

In summary, while there have been several studies that suggested a beneficial role of folic acid in decreasing OFC risk, results are often mixed in terms of the estimated effects of folic acid as well as whether CL/P or CP or both are affected. This is in part due to differences in the studied dose and definition of folic acid supplements (multivitamins, folic acid supplements, or both), measurement and sample selection biases, and statistical models including adjustment for confounders.

Folic Acid Fortification and Oral Clefts

A few countries have introduced folic acid fortification of grain and flour given the strong evidence for the preventive effect of folic acid on NTDs. Indeed, this evidence and its subsequent application to populations are considered to be one of the major public health successes in the field of birth defects. Unlike the case for NTDs, there is no converging evidence for significant changes in birth prevalence for oral clefts post folic acid fortification. In the United States, where folic acid fortification of grain products was mandated on January 1, 1998, three studies reported non-significant reductions in CL/P prevalence of 3% in Texas (Hashmi et al., 2005), 5% in 23 states reporting to the National Birth Defects Prevention Network (Canfield et al., 2005), and 14% in Arkansas (Simmons et al., 2004), post fortification. Canfield et al. (2005) reported a significant 12% reduction in CP prevalence. A recent study reported a significant 6% reduction in OFC prevalence based on birth certificate data from 45 states from 1990 through 2002 (Yazdy et al, 2007).

Ray et al. (2003) reported a slight non-significant increase in the prevalence of OFC after two years of fortification of cereal grain products (1998 through 2000) in Ontario, Canada.

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Also, in an evaluation of the effects of fortifying wheat flour with folic acid in Chile starting 2000, Castilla et al. (2003) reported no significant changes in prevalence of OFC, while a significant reduction of 31% in NTDs was shown (Lopez-Camelo et al., 2005). In a meta-analysis of fortification studies in the United States and Canada, Johnson and Little (2008) estimated a reduction of about 7 and 8% in the prevalence of CL/P and CP respectively.

Longer periods may be required for a more comprehensive evaluation of potential changes in prevalence of OFC post fortification. However, given the evidence of NTD reduction of up to 50% in similar periods and across multiple populations (e.g. Lopez-Camelo et al., 2005; Canfield et al, 2005; Williams et al, 2005; Liu et al, 2004; De Wals et al, 2007, 2008), these results suggest that low doses of folic acid may be inadequate to prevent occurrence of OFC as also suggested by other studies (Czeizel et al, 1999; Czeizel, 2004). Further, the studies of changes in prevalence over time suffer from limitations including potential confounding by other simultaneously changing relevant factors and the lack of well-matched control groups.

Interventional Studies of Folic Acid and Oral Cleft Recurrence

Only a handful of interventional studies have been conducted over the last 50 years to study the effect of folic acid supplementation on recurrence of oral clefts in mothers with a child with OFC. The decrease in OFC recurrence among the folic acid groups reported in these studies, independent of statistical significance, ranges from about 24 to 100%. Conway (1958) reported no recurrent cleft cases among 59 births to mothers with history of OFC in previous births who received a multivitamin that included 0.5 mg of folic acid. The recurrence rate in a group of 78 births to mothers who did not receive the supplement was 5.1%. Peer et al. (1964) reported a 53% reduction in the recurrence of OFC in a group of 176 women who received a multivitamin in addition to 5 mg folic acid and 10 mg vitamin B6 during the first pregnancy trimester, compared to a control group of 418 mothers ($p=0.1$). In an extended study of Peer et al. (1964) with more supplemented women, Briggs (1976) reported a 35% reduction in recurrence of OFC ($p=0.2$), but a 65% reduction in CL/P recurrence ($p=0.06$). Tolarova (1982) reported an 84% reduction in recurrence of CL/P in a group of 80 women who received a multivitamin and 10 mg of folic acid during three months before and after pregnancy ($p=0.02$), compared to a control group of 202 women. Using data on a larger sample that included women with CL/P (40% of intervened sample) and mothers of a child with CL/P, and the same intervention as Tolarova (1982), Tolarova and Harris (1995) reported a 66% reduction in recurrence of CL/P ($p=0.03$). Johnson and Little (2008) estimated a significant 67% reduction in CL/P recurrence based on these studies. These calculations are primarily descriptive given the array of interventions and populations used, but from an exploratory perspective, may be helpful for gauging expected treatment effects of folic acid to form hypotheses in clinical trials. The results of these studies are suggestive of potential preventive effects of high dose folic acid on cleft recurrence.

Interventional Studies of Folic Acid and Oral Cleft Occurrence

The Hungarian randomized and cohort controlled trials of the multivitamin intervention (Czeizel and Dudas, 1992; Czeizel et al., 2004) support the notion of lack of preventive effects of low doses of folic acid on occurrence of oral clefts (czeizel et al, 1999; Czeizel, 2004). These trials found no statistically significant effects on CL/P and CP (Czeizel, 2004).

Other Studies

Other studies of micronutrient and folate exposures have also suggested associations with oral clefts in humans. Rouget et al. (2005) reported a reduction in OFC risk with a sufficient folate diet (around 0.35 gm daily) in a French sample (Rouget et al, 2005). Van Rooij et al.

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(2003) reported low maternal post pregnancy B12 levels and low infant serum folate among infants affected with OFC.

Hernandez-Diaz et al. (2000) reported that exposure to folic acid antagonists early doubled the risks of OFC. Animal studies also provide support for anti-teratogenic effects of folic acid supplementation and dietary folate on OFC including studies in mice, rats and dogs (Peer et al.,1958; Reynolds et al., 2003; Bienengraber et al, 2001; Malek et al, 2003; 2004, Paros and Beck, 1999; Fu et al, 1996; Burgoon et al, 2002; Elwood and Colquhoun, 1997). These studies also provide suggestive results for a potential role of folic acid and possibly other micronutrients in OFC etiology/prevention.

Folate Gene Interaction Studies

Interactions between vitamin use and the folate metabolic pathway have also been intensively studied. Genes that code for folate metabolizing enzymes, such as Methylenetetrahydrofolate reductase (*MTHFR*), are optimal candidates for gene-folic acid interaction studies. Specific alleles in these genes, such as the T677C of *MTHFR*, may modify the effects of folic acid supplementation. Main candidate genes for interaction studies include *MTHFR*, *MTHFD*, *MTR*, *MTRR*, *RFC1*, GCP2, *CBS*, *BHMT*, *BHMT2* and *TS*.

There are numerous and often contradictory studies for the MTHFR T677C variant (Blanton et al., 2002; Jugessur et al., 2003; Van Rooij et al, 2003; Gaspar et al., 2004; Vieira et al., 2005; Verkleij-Hagoort et al., 2007; Chevrier et al., 2007; Boyles et al., 2008; Mills et al., 2008). Shelnut et al. (2003) reported that changes in folate and homocysteine levels with an increase in dietary folate varied by *MTHFR* 677 status. A potential interaction between vitamin use and *RFC1* has also been suggested (Shaw et al., 2003; Vieira et al., 2005), though no evidence has been observed in a recent study (Pei et al, 2006). In sum, there is as yet little consensus among the many studies of interaction between vitamin/folic acid use and genetic factors in the etiology of OFC.

Limitations of Previous Studies

The studies described above are suggestive of protective effects of folic acid supplementation on OFC risks, especially for CL/P, but they all suffered from data and design limitations. The interventional studies for human recurrence have serious limitations, particularly in lacking randomized assignment into treatment and control groups and in using interventions that combine folic acid with other supplements and prevent the identification of the effects of folic acid (Czeizel, 2002). The non-random assignment introduces the biases of self-selection into the treatment, which may confound the study results and introduce differences in outcomes between the treated and untreated groups that are not a result of the treatment. Most of the previous interventional studies also suffered from small sample size and power limitations.

Observational case-control studies also suffer from the problems of non-random self or provider selection of supplement use. The use of multivitamins and folic acid supplements during pregnancy is in part determined by perceived health risks that may also affect the risk for OFC and other birth outcomes (Wehby et al, 2009). Specifically, women with unfavorable pregnancy histories or health problems may use more folic acid supplements but may also have a greater risk for adverse pregnancy outcomes including births defects such as OFC. Confounding bias in observational studies also results from the lack of data on health behaviors that may be correlated with both supplement use and OFC. Other limitations include potential bias in self-reported use of supplements (both recall bias as well as biased report of use based on the pregnancy outcome such as OFC status in studies that

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measure use after pregnancy, which are the majority of studies in this area) and the limited data on the folic acid content/dose and duration/intensity of use. Only double-blinded randomized clinical trials (RCTs) with sufficient sample sizes can provide the opportunity to clearly identify the true preventive effects of folic acid.

Clinical Trials for Recurrence

The NTD model showing preventive effects of high and low dose folic acid on recurrence and occurrence respectively, and the suggestive results from interventional studies and observational studies for preventive effects of high doses on recurrence and occurrence of OFC (summarized above) strongly indicate that large doses of folic acid are best suited for evaluation in RCTs of recurrence. The Oral Cleft Prevention Program (OCPP) was developed over the past eight years as a double-blinded RCT to estimate the effect of periconceptual supplementation with high dose folic acid (4 mg per day), which proved effective in preventing recurrences of NTDs (MRC, 1991), versus low dose (0.4 mg per day) on prevention of CL/P recurrence among women who have CL/P or who have had a child with CL/P. The study was initiated under the sponsorship of the NIDCR, NICHD and the Gates Foundation, and is currently funded by the NIDCR. The OCPP has established an important infrastructure to implement a large-scale RCT to study the role of high dose folic acid in prevention of CL/P recurrence including building protocols for treatment provision and outcome measurement, data collection instruments, data management systems and quality-control procedures. The OCPP currently involves multiple craniofacial clinics in Brazil including the Hospital de Reabilitação de Anomalias Craniofaciais (Centrinho) in Bauru, Hospital de Clinicas de Porto Alegre (HCPA) in Porto Alegre, Hospital Santo Antônio- Centrinho-Obras Sociais Irmã Dulce (OSID) in Salvador, Instituto Materno Infantil de Pernambuco (IMIP) in Recife, Centro de Atendimento Integrado ao Fissurado Lábio Palatal (CAIF) in Curitiba and the Fundação para reabilitação das deformidades crânio-faciais (FUNDEF) in Lajeado. RTI International maintains the Data Center responsibilities for data management and storage.

There is a tremendous need for a double-blinded RCT, such as the OCPP, in order to identify the effects of folic acid on recurrence of OFC. The double blinded randomized design will separate the effect of the intervention from the confounding effects that are inherent in the interventional and observational designs of previous studies. This design will also address a fundamental challenge in the clinical care of families with one or more individuals with a cleft; that is, how to manage recurrence prevention. Reducing the recurrence of OFC is expected to have important reductions in the quality of life and economic costs of OFC at the individual, family and societal level.

Discussion and Conclusions

There is some suggestive evidence for a possible role of folic acid in prevention of OFC. However, several important questions remain unanswered including confirming whether folic acid prevents OFC, whether it prevents occurrence or recurrence or both, whether it prevents CL/P, CP or both, and identifying whether low or high doses are effective for prevention. Studies to date have provided mixed results particularly in regards to whether low or high dose folic acid can prevent primary occurrence. Most case-control observational studies indicating a preventive effect are likely to have evaluated low to moderate doses of (<1mg) folic acid though the majority did not measure or report the dose. One observational study found a decrease in CP with high dose folic acid, though no significant effect on CL/P (Czeizel, 2004). The evidence is also mixed for the effects on OFC type. The treatment self-selection and confounding biases in addition to sample selection biases and measurement errors are likely the primary contributors to differences in results. Given that folic acid has been shown to prevent NTDs across different populations, it is unlikely that any potential

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real effect of folic acid on prevention of OFC varies significantly across populations, though this remains to be identified in future studies.

Given that low doses of folic acid are known to prevent NTDs, it would not be possible to conduct a randomized clinical trial to study the effects of low dose folic acid on occurrence of OFC as a placebo control group would be unethical. Conducting an RCT to study the effects of high dose folic acid on OFC occurrence might not be the first research priority at this stage given that low doses have not been ruled out to be ineffective for occurrence. Research should be focused on improving the quality of the observational studies on OFC occurrence including the use of larger and more representative samples and better measurement of folic acid use including timing, dose, and intensity of use as well as use of other dietary supplements that should be accounted for. The specification of analytical models can also improved, including better measurement and accounting for confounders that are related to both folic acid use and OFC risks including nutrition, maternal health risks, family history of birth defects, health behaviors, demographic and socioeconomic characteristics. Instrumental variable analyses with genetic instruments can also be employed to assess the effects of blood folate levels on OFC while accounting for unobserved factors that determine self-selection into folate supplement use and dietary patterns (Wehby et al., 2008). Further, meta-analyses of observational studies [such as Badovinac et al. (2007) and Johnson and Little, (2008)] should continue to be conducted to obtain improved estimates of the average effects of folic acid on OFC occurrence.

The strong evidence of high dose folic acid in preventing the recurrence of NTDs (MRC, 1991) and the preliminary evidence from the non-randomized interventional studies of OFC recurrence suggest that identifying the effects of high dose folic acid on OFC recurrence is a high research priority. The effects of high dose folic acid on OFC recurrence can be identified through a double-blinded RCT design. The relative low rate of OFC recurrence (about 5%) implies that a large sample of births is needed to have adequate statistical power to identify the effect of folic acid. Specifically, about 1,580 total births are required for a one-sided hypothesis of 50% reduction in a baseline recurrence risk of 5%. Therefore, multi-site international collaborative efforts are needed to successfully conduct an RCT trial for OFC recurrence. As a reference, the MRC (1991) trial for NTD recurrence was conducted over a course of about 8 years in 33 centers in 7 countries.

The OCPP is a model RCT for OFC recurrence that can be extended to multiple sites worldwide. The study has enrolled eligible at risk women, regardless of whether they are planning a pregnancy or not over their years of participation in the study, in order to estimate the treatment effectiveness (overall population effect). However, this introduces the challenge of enrolling a much larger sample of study subjects than the minimum required number of live births, since only a small percentage of subjects may become pregnant during the study period. An alternative approach, similar to the MRC, involves enrolling at risk women who are planning on becoming pregnant over the next year or two after enrollment. This design requires fewer resources but will estimate an effect that is specific to the group who are planning their pregnancy. This tradeoff represents a real challenge for an RCT to study OFC recurrence. However, given that the women who are planning their pregnancy will likely be the primary group who will utilize high dose folic acid if found to be effective in reducing recurrence, estimating the treatment effects based on this group seems appropriate as the estimates can be generalized to a large group of the at risk population of women, especially when the large constraints of the alternative study sample are considered. The OCPP can be developed as a multi-country multi-site study with a more focused recruitment model that limits enrollment to women who are planning on becoming pregnant in order to obtain the required number of births with reasonable resources and timelines.

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Finally, other micronutrients have also been implicated in OFC though the evidence remains smaller than that for folic acid. B1 and B6 deficiencies were also associated with an increased risk of OFC (Krapels et al., 2004a; Munger et al., 2004; Tamura et al., 2007) as were myo-inositol and zinc (Krapels et al., 2004b; Tamura et al., 2005), though no effects of Zinc levels on OFC have recently been reported in a sample from the United States (Munger et al., 2009). While these micronutrients could also be considered in other RCTs, the case for folic acid alone is far more compelling.

Acknowledgments

George Wehby acknowledges funding from the NIDCR (1R03 DE018394) and the CDC (1R01DD000295). Dr. Murray acknowledges funding from the NIDCR (U01 DE-017958, R01 DE-08559, and P50 DE-16215).

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