Short Communication

Whole blood propionylcarnitine in newborns with orofacial cleft

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Abstract

Orofacial clefts are thought to be determined by the interplay of genetic and environmental factors. Experiments on animals demonstrated that vitamin B12 supplemented diets antagonize selected teratogens during palatogenesis. Increased propionylcarnitine in neonates is regarded as a marker of maternal vitamin B12 deficiency. The retrospective study was undertaken to determine whether increased propionylcarnitine in newborns is associated with orofacial clefts. Fifty-two newborns with isolated cleft lip with or without cleft palate (CLP) and 107 control newborns without congenital anomalies were investigated. Whole blood propionylcarnitine concentrations were measured using tandem mass spectrometry. The mean concentrations of propionylcarnitine in newborns with clefts and controls were $2.82 \pm 1.06 \mu\text{mol L}^{-1}$ and $2.68 \pm 0.94 \mu\text{mol L}^{-1}$, respectively. *T*-test for equality of means did not confirm any significant differences between both groups ($P = 0.381$). Deficiency of vitamin B12 with metabolic disturbances seems not to be a risk factor for CLP in the investigated group of patients.

Keywords: cleft palate, vitamin B12, propionylcarnitine.

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Isolated (nonsyndromic) cleft lip with or without cleft palate (CLP) represent a significant proportion of all birth defects. They affect approximately 1 in 500–3000 newborns. A number of hypotheses have been proposed to explain the pathogenesis of CLP (Krapels *et al.* 2006; Weingartner *et al.* 2007). Experimental studies showed that vitamin B12 (cobalamin) antagonize selected teratogens during palatogenesis in rodents (Natsume *et al.* 1986; Elmazar *et al.* 1992; Lu *et al.* 2008). Polymorphisms in genes related to vitamin B12 metabolism in mother and/or her offspring seem to be linked with increased risk of giving birth to the child with CLP (Mostowska *et al.* 2006; Weingartner *et al.* 2007; Fox & Stover 2008). In humans, maternal multivitamin use seems to reduce the risk of nonsyndromic orofacial clefts in the offspring (Itikala *et al.* 2001; Weingartner *et al.* 2007). For humans, the only

source of vitamin B12 (other than supplements) is foods of animal origin. Results of studies on periconceptional dietary intake of cobalamin or supplementation with small doses of the vitamin and occurrence of orofacial clefts are conflicting (Krapels *et al.* 2004; Bille *et al.* 2007; Wang *et al.* 2009).

Vitamin B12 participates as a cofactor in two metabolic reactions (Green 1995). In the cytosolic reaction, cobalamin is required in the folate dependent methylation of homocysteine to methionine (CH_3 -homocysteine). Hyperhomocysteinemia is regarded as a risk factor for orofacial clefts and other structural malformations like spina bifida and conotruncal heart defects (Wong *et al.* 1999; Shaw *et al.* 2006). Moreover, the study by Shaw *et al.* (2006) delivered some evidence of decreased risk for clefts with increasing periconceptional intake of methionine. In mitochondria,

vitamin B12 is required for the enzyme methylmalonyl-CoA mutase, which decreased activity results in accumulation of methylmalonyl-CoA and propionyl-CoA (Deodato *et al.* 2006). It was reported that excess of propionyl-CoA in vitamin B12 deficiency is converted to propionylcarnitine (C3) (Brass & Stabler 1988; Kushnir *et al.* 2002; Campbell *et al.* 2005; Marble *et al.* 2008). High levels of propionylcarnitine may serve as a marker of vitamin B12 deficiency and there are published reports on newborns nutritional shortage of vitamin B12 secondary to maternal deficiency detected by acylcarnitine profiling through newborn screening for inborn errors of metabolism (Campbell *et al.* 2005; Marble *et al.* 2008). Currently, propionylcarnitine assessment is included in all tandem mass spectrometry (MS/MS)-based newborn screening programmes (Campbell *et al.* 2005).

To investigate the hypothesis of the involvement of vitamin B12 deficiency in abnormal palatogenesis, we studied the newborn screening results for propionylcarnitine in children with CLP.

Study population and methods

Study population

All patients with isolated CLP attending our institution and unrelated healthy children without congenital anomalies of similar age attending three local primary care pediatricians were considered for inclusion for the study. Inclusion criteria were as follows: (1) singleton pregnancy; (2) gestational age at delivery ≥ 36 weeks and/or birth weight > 3000 g, which have long been recognized as important determinants of newborn health; and (3) delivery in the years 2004–2007 in hospitals located on area covered by the MS/MS Newborn Screening Program provided by our institution. Case eligibility was ascer-

tained from detailed medical records. Finally, we performed retrospective analysis of propionylcarnitine concentration in 52 newborns with isolated CLP (cleft group) and 107 healthy newborns without congenital anomalies (control group). Slightly more case newborns were males (67%) compared with the control group (62%), $P > 0.05$. Mean gestational age at delivery of cases and controls were the same (39 weeks). Birth weights of orofacial cleft newborns were, in mean, 150 g lower than that in not affected children (3390 g vs. 3540 g, $P = 0.05$). All participants were white Poles. The study protocols were approved by the local ethics committee.

MS/MS analyses

The dried blood spots ('Guthrie cards') were generally collected 3 days after the infant's birth. Discs were punched from the areas of dried blood on a filter paper. Specimens were extracted in pure methanol containing known concentrations of stable isotopically enriched amino acids and acylcarnitines. MS/MS analyses of the acylcarnitines as butyrate esters were performed on tandem mass spectrometer (SCIEX Api 2000, Concord, Canada) configured with liquid chromatography for sample handling. The working range was $0.01\text{--}50.0 \mu\text{mol L}^{-1}$ of propionylcarnitine.

Statistical methods

The results were analysed using commercially available software packages (PASW Statistics Base, SPSS Polska Sp. Z.o.o., Krakow, Poland) Statistical methods for propionylcarnitine concentration analysis as continuous variable included Spearman correlation analysis and the *t*-test. Significance was defined as a *P*-value equal or less than 0.05.

Key messages

- Deficiency of vitamin B12 is a potential, but little researched risk factor for orofacial clefts. High levels of propionylcarnitine may serve as a marker of vitamin B12 deficiency.
- Severe deficiency of vitamin B12 with increased propionylcarnitine seems not to be a risk factor for cleft lip with or without cleft palate in the investigated group of Polish newborns.
- The experiences from tandem mass spectrometry-based newborn screening programs might throw a new light on biochemical mechanisms of abnormal palatogenesis in humans.

Results

The mean concentrations of whole blood propionylcarnitine in newborns with CLP and controls were $2.82 \mu\text{mol L}^{-1}$ (range 1.00–5.56; SD 1.06) and $2.68 \mu\text{mol L}^{-1}$ (range 0.87–5.38; SD 0.94), respectively, *t*-test for equality of means did not confirm any significant differences between both groups ($P = 0.381$). There were no significance correlations between the level of propionylcarnitine and clinical variables such as birth weight, $r = 0.07$ and gestational age at delivery, $r = -0.11$ (all $P_s > 0.05$).

Discussion

To our knowledge, the present report is the first study investigating propionylcarnitine concentrations in patients with isolated structural malformations. We found no association between newborn whole blood propionylcarnitine level and the risk of CLP. The results suggest that deficiency of vitamin B12 is not involved in abnormal palatogenesis in the investigated group of children. However, data presented by Kushnir *et al.* (2002) suggests that propionylcarnitine may be increased in severe vitamin B12 deficiency but cannot reliably detect early stages of this disorder. The propionylcarnitine in newborn screening may not be sufficient to rule out all the cases of cobalamin deficiency, however, MS/MS has enabled expansion of population screening for the vitamin deficiency (Campbell *et al.* 2005; Marble *et al.* 2008). On the other hand, while conducting further studies we should keep in mind that methylmalonic acidemia with associated increased propionylcarnitine may result not only from acquired vitamin B12 deficiency but also from inborn errors of metabolism (Deodato *et al.* 2006; Marble *et al.* 2008).

So far, very few studies have investigated vitamin B12 concentrations in mothers or affected children in relation to orofacial clefts (Stoll *et al.* 1999; Wong *et al.* 1999; van Rooij *et al.* 2003). The study by van Rooij *et al.* (2003) demonstrated that around 14 months after delivery a low vitamin B12 concentrations in mothers, but not in index children, linked with increased risk of orofacial clefts. The molecular mechanisms of abnormal palatogenesis are poorly understood. The rela-

tively high and fluctuating frequency of orofacial clefts is attributable to the sensitivity of facial development to environmental insult (Krapels *et al.* 2006; Weingartner *et al.* 2007). There are studies suggesting a link between one-carbon metabolism and clefting in humans (Mostowska *et al.* 2006; Fox & Stover 2008). However, the study and control groups originated from mostly omnivorous population and vitamin B12 deficiency is regarded by some investigators as less likely to be the cause of orofacial clefts, because it is difficult to induce in omnivorous women in childbearing age (Weingartner *et al.* 2007). Presented negative results on propionylcarnitine do not exclude the potential role of vitamin B12 and disturbed methionine metabolism in the aetiology of CLP.

Our results might be influenced by the sample size, which may not be large enough to detect modest differences of propionylcarnitine level in the study and the control groups. Therefore, the exclusion of the association between vitamin B12 level in neonate and the risk to be born with cleft requires further studies of metabolic profiles, which should be performed in larger groups of affected children.

There are some other limitations to consider. The assessments of propionylcarnitine were undertaken only in newborns, which may have diminished the possibility of finding teratologically pertinent fetomaternal changes. The study design has retrospective nature and we lacked the ability to access additional information on newborn feeding and maternal diet.

This study has also some notable strengths. The investigated ethnically homogeneous, mostly omnivorous population was from an area where preconceptual multivitamin use is low (2.8–6.4%) (Hozyasz *et al.* 2009). Data of propionylcarnitine assessments were from population based MS/MS Newborn Screening Program, but we were able to establish phenotypic cleft type in all cases.

In conclusion, this is the first report of propionylcarnitine level in newborns with orofacial cleft. Severe deficiency of vitamin B12 seems not to be a risk factor for CLP in the investigated group of patients. It is important to stress the need for further studies of metabolic profiles of affected children, which might throw a new light on the nutritional intervention strategies to reduce risk of orofacial clefts.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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