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Association of TAS2R38 variants with sweet food intake in children aged 1 to 6 years

Ingrid Pawellek[1], Veit Grote[1], Peter Rzehak[1], Annick Xhonneux[2], Elvira Verduci[3], Anna Stolarczyk[4], Ricardo Closa-Monasterolo[5], Eva Reischl[6], Berthold Koletzko[1], European Childhood Obesity Trial Study Group

[1] Dr von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Lindwurmstrasse 4, 80337 Munich, Germany

[2] CHC St Vincent, rue François Lefèbvre, 207, 4000 Liège-Rocourt, Belgium

[3] Department of Pediatrics, San Paolo Hospital, University of Milan, Via Antonio di Rudinì, 8, 20142 Milano, Italy

[4] Children's Memorial Health Institute, Dept. of Gastroenterology, Hepatology and Pediatrics, Al. Dzieci Polskich 20, 04-730 Warsaw, Poland

[5] Pediatrics Research Unit, Universitat Rovira i Virgili, IISPV, C/ Sant Llorenç 21, 43201, Reus, Spain

[6] Research Unit of Molecular Epidemiology and Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

Correspondence:

Berthold Koletzko MD PhD Dr h c, Professor of Paediatrics

Department of Metabolic Diseases and Nutrition

Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich

Lindwurmstraße 4, 80337 München, Germany

Telefon: +49 89 4400 52826 , Fax: +49 89 4400 57742

Email: office.koletzko@med.uni-muenchen.de

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Abstract

We aimed at studying whether genetic variants of the TAS2R38 gene are associated with energy intake from sweet tasting foods, total energy and macronutrient intake and body weight in children.

Children (n=691) from five European countries were genotyped for the first variant site rs713598 of the TAS2R38 bitter receptor gene. Three-day dietary records were obtained yearly from one to six years of age. Foods were categorized in sweet and non-sweet-tasting. Mixed models were used to describe group differences in food and nutrient intake and BMI z-score over time.

TAS2R38 genotype was related to energy intake from sweet tasting foods: Children with PP and PA genotype consumed an average 83 kJ/d (95% CI 21 to 146; p=0.009) more sweet tasting foods than children with AA genotype and a mean 56 kJ/d (95% CI 15 to 98; p=0.007) more energy from energy dense sweet products. Intake of sweet tasting foods was lower in girls than boys and differed between countries. TAS2R38 genotype was not associated with the intake of energy, macronutrients, sugar, single food groups and BMI z-score.

Despite many other factors influencing food preference and intake in children, actual intake of sweet food items is associated with TAS2R38 genotype. Children with PP or PA genotype consume more (energy dense) sweet tasting foods.

Introduction

Obesity and the metabolic syndrome has become one of the greatest challenges for health care systems, with high ensuing costs in industrialised countries. A recent study showed a prevalence of overweight preschool children aged 4 to 7 years in the range of 7.6% (girls in Germany) to 29.8% (girls in Spain), based on the WHO criteria (van Stralen et al, 2012). Obesity is the result of an impaired energy balance with too high energy intake relative to energy expenditure. 7 to 14 year-old US children consume 46% of their total energy intake via discretionary dietary fat and added sugar (Brady, Lindquist, Herd & Goran, 2000); thus these dietary components have a high contribution to energy intake without delivering valuable nutrients. Children's food preferences are influenced by genetic, environmental and educational factors (Scaglioni, Arrizza, Vecchi & Tedeschi, 2011). Numerous studies confirmed that obesity is strongly influenced by a genetic predisposition (Keller, Pietrobelli, Must &Faith, 2002) and genetics may also play a role in the development of food preferences and dietary habits (Scaglioni, Arrizza, Vecchi & Tedeschi, 2011). The WHO proposed in their recently released guidelines to reduce intake of free sugars to 5% of total energy per day because of the effects of high sugar intake on body fat deposition, adiposity and dental caries (World Health Organisation [WHO], 2015). The TAS2R38 gene encodes a seven-transmembrane G protein-coupled receptor for the perception of glucosinolates, bitter-tasting phytochemicals in Brassica vegetables (Kim & Drayna, 2005). Individual variation in the sensitivity to taste the two bitter compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), chemical compounds related to the glucosinolates can partially be explained by the genetic variation of the TAS2R38 receptor (Kim & Drayna, 2005). Outside of Africa mainly two allele forms can be found that differ at three nucleotide positions in the gene and in three amino acids in the receptor protein (A49P, A262V, and V296I) (Kim &

Drayna, 2005). The amino acid combination PAV represents the taster variant (49%) and AVI (47%) the non-taster variant (Kim & Drayna, 2005).

In numerous investigations, tasters showed lower acceptance of cruciferous, green and raw vegetables (Drewnowski, Henderson, Hann, Berg & Ruffin, 2000; Kaminski, Henderson & Drewnowski, 2000; Yackinous & Guinard, 2002), whereas a recent study found no association between genetic variations of the TAS2R38 gene and the intake of brassica vegetables (Gorovic et al., 2011). Intake of sweet tasting food or sugar intake was also discussed to be associated with bitter taste sensitivity. Some studies reported that taster children had a higher intake of sugar and sweet tasting food (Mennella, Pepino & Reed, 2005; Keller & Tepper, 2004; Joseph, Reed & Mennella, 2016), while others did not find relationships (Keller et al., 2010; Keller et al., 2014)

Furthermore some studies hypothesized that PROP taste sensitivity is associated with sensitivity to other bitter tastes, sweet taste, the pungency of chili peppers, the astringency of alcohol, and the texture of fats (Tepper et al., 2009). We hypothesized that children with the TAS2R38 PP or PA taster genotype have a higher actual intake of sugar and sweet tasting food to compensate for the bitter taste and other taste sensitivities. The aim of our study was to examine whether TAS2R38 genotype is associated with intake of total energy, energy from sweet tasting foods, sugar, carbohydrate, fat, protein, single food groups and BMI z-score in European children.

Material and Methods

The analysis was performed in the framework of a double-blind, randomized, multicentre intervention trial in Germany, Belgium, Italy, Poland, and Spain on early protein intake and early growth (Koletzko et al., 2009) and adiposity at six years of age (Weber et al., 2014). All participants were apparently healthy and term infants who were born from uncomplicated, singleton pregnancies (Koletzko et al., 2009).

Bitter receptor

Buccal cells were collected on filter cards (IsoCode Cards of Schleicher and Schüll, Dassel, Germany) or on FTA Indicator Cards (Whatman, Middlesex USA) during study visits. DNA extraction from the cards was accomplished by a washing procedure according to protocols provided by the manufacturers.

The polymerase chain reaction (PCR) and genotyping analysis of the TAS2R38 gene was performed in the Helmholtz Zentrum München using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Individuals were grouped by the first SNP rs713598 resulting in an allele change on base pair 145 from G(uanine) to C(ytosine) which causes an amino acid change on codon 49 from A(lanine) to P(roline). TAS2R38 genotypes PP and PA are grouped together, as they represent the taster variant, while AA represent the non-taster variant (Kim & Drayna, 2005).

Dietary intake assessment

Parents completed three-day, weighed dietary records on three days (1 weekend day and 2 week days) at the ages of 12, 24, 36, 48, 60 and 72 months. For evaluation a trained dietician validated the protocols, clarified open issues with the parents and transferred the dietary records in the database in each study centre via a dedicated software program (Verwied-Jorky S et al., 2011).

The German BLS 3.01 (Bundeslebensmittelschlüssel Version 3.01) formed the basis for calculation of nutrient intakes from food intake data in all participating countries. Food items not found in BLS were added by the dieticians evaluating the dietary records (custom food items). Standard operating procedures were developed and implemented for the introduction of the dietary records in the program to harmonize procedures (Verwied-Jorky S et al., 2011). For custom food items, information on nutrient content was provided from manufacturers. Mono- and disaccharides were considered as sugars. If data on sugar content was not available it was estimated based on comparison with similar products. Food items were categorized into subgroups according to food composition and taste and divided into sweet and non-sweet tasting. In a first step this was done by a qualified dietician, in further steps the categorization was reworked by other dieticians, especially for custom food items in each study centre. In case there was no consensus, the food item was categorized in 'not sweet'. Sweet tasting foods comprise pastry products, sweet tasting beverages (fruit juice, soft drinks, fruit drinks), sweetened cereals (sugar >10g/100g), desserts, fruit and fruit products, sweetened dairy products, sweets, instant cacao powder, sugar and honey and sweet main dishes.

Fruit and fruit products contain fresh and dried fruits as well as fruit mash or preserves and jam. For further analysis sweet tasting food items were divided into sweet products with high energy density (energy density ≥ 200 kcal/100g) and sweet products with low energy density (energy density < 200 kcal/100g).

Anthropometrics and other explanatory variables

Body weight and other anthropometric variables were evaluated yearly from 12 to 72 months of age. All study centres used the same equipment for measuring body weight (Seca 336 scales at ≤24 months and Seca 702 scales at ≥ 24 months; Seca, Hamburg, Germany).

Mother's education, mother's age at child's birth and child's birth order was reported at study entry. Mother's height and weight was measured during study visits. Mother's education was categorized in high, middle and low by the level of graduation. Children's feeding type was categorized into three groups: higher or lower protein formula group (randomized groups) and breastfed children.

Statistical analysis

Our primary endpoint was the total daily energy intake from sweet tasting foods (kJ/d). Secondly, we investigated the association of TAS2R38 genotype with the average daily intake of energy from sweet products with high energy density (kJ/d), energy from sweet products with low energy density (kJ/d), carbohydrates (g/d), sugar (g/d), fat (g/d), protein (g/d), total energy (kJ/d), energy intake from single food groups (kJ/d) and BMI z-score.

Differences between TAS2R38 genotypes in the distribution of categorical variables were tested by a chi-square test or Kruskall-Wallis test as appropriate. Mixed linear (growth) models with random intercept and random linear slope (age) and fixed quadratic and cubic age terms were used to describe group differences in food and nutrient intake as well as BMI z-score from 12 to 72 months of age. We adjusted for gender and country. Additionally all models looking at the effects on macronutrient and sugar intake were adjusted for total energy intake; models with BMI as the outcome were also adjusted for early feeding type (formula/breastfeeding). Effects of potential confounders like single mother status, mother’s education attainment, marital status, maternal age at birth, birth order, and maternal smoking were assessed. In a second step we additionally adjusted for current BMI and energy misreporting status in a subset in which weight and height at the specific time point was available. We calculated misreporting based on the ratio of mean energy intake to energy requirements. Energy requirements were based on Butte (12 months) and

Torun (≥24 months) using regression functions including weight (Butte NF, 2005; Torun B, 2005); energy needed for tissue disposition of growth was added. Individual normal ranges of the ratio were defined according to the method described by Black & Cole (2005); ratios below and above the normal range were defined as under- and over-reporters, respectively, while all others were defined as normal reporters.

The following equation specifies the estimated mixed model formally:

$$Y_{it} = \beta_{0i} + \beta_{1i} \times AGE_{it} + \beta_2 \times AGE_t^2 + \beta_3 \times AGE_t^3 + \beta_3 \times genotype + \beta_{4i} \times (AGE_{it} \times genotype) + \beta_5 \times gender + \beta_6 \times DE + \beta_7 \times PL + \beta_8 \times IT + \beta_9 \times BE + e_{it}$$

where Y_{it} is intake (kJ) for subject i at age t

$ß_{0i} = ß_0 + u_i$ is fixed effects intercept plus individual deviation u_i for person i

$ß_{1i} = ß_1 + v_i$ is fixed effects linear slope plus individual deviation v_i for person i

$ß_2$ is fixed effects quadratic slope for all children

$ß_3$ is fixed effects cubic slope for all children

$ß_{4i}$ is interaction effect of linear age slope and genotype plus individual deviation for this interaction effect

$ß_5$ is fixed effects of gender for all children

$ß_6$ to $ß_9$ are fixed effects of study countries (GE=Germany, BE=Belgium, IT=Italy, PL=Poland; reference group are study children from Spain)

e_{it} is individual error for child i at age t.

A fixed effect can be interpreted as the population average effect and a random effect as the individual deviation of that average effect. In this longitudinal model $ß_0$ gives the average outcome at age 12 months and the u_i are the individual deviations from that outcome at that age (e.g. Individual different energy intake from sweet tasting foods). The estimate $ß_1$ gives the average linear change in the outcome and v_i is the individual deviation from this average change for the outcome (e.g consuming

more energy from sweet tasting foods at a given age). The other age related effects are specified as fixed effects and that means: Although the outcome changes individually with a different velocity (linear random slope) resulting in individually different outcome trajectories the quadratic or cubic change is not allowed to individually vary.

Missing data in modern methods like multilevel modelling are assumed to be missing at random (MAR). Multilevel models are maximum likelihood based methods and thus evaluates the entire joint distribution of the responses (the repeated outcomes over time) and thus yield valid estimated if missing data are MAR (Fitzmaurice G.M., Laird N.M., Ware J.H., 2004). In contrast, analysis based only on complete data are assumed to be invalid (Twisk J.W.R., 2003).

Dietary records with energy, fat, carbohydrate or protein intake 3 SD above or under the mean intake for each respective country by time point were excluded. Accordingly, we excluded for the effect estimates of TAS2R38 genotype on energy intake from sweet tasting foods 29 values of 24 subjects with > 3 SD above or below the mean of energy intake from sweet tasting foods.

Overall, there were 3156 observations from 691 children with TAS2R38 genotype and nutritional information; there were 2603 observations from 684 children with BMI measurement and 2548 observations from 675 children with additional misreporting status.

Data management and statistical analyses were carried out with the software packages SPSS Statistics 21 (SPSS Inc, Chicago, IL) and Stata version 13.1 (StataCorp LP, College Station, TX).

Results

TAS2R38 genotype and dietary data for at least one time point between 12 and 72 months was available in 691 children (444 or 64.3% genotype PP or PA, 247 or 35.7% genotype AA). There were slight but non significant differences in the distribution of genotype between countries (CHI square test, p=0.586): Spain (67.7%) and Poland (60.1%). The number of children with a food protocol decreased from 574 (64.6% tasters) at 12 months of age to 382 (64.4% tasters) at 72 months.

Study population

Table 1 shows characteristics of our study population with at least one valid three-day dietary record between 12 and 72 months of age. Distribution of TAS2R38 genotype did not differ by any of the displayed characteristics except single mother status: There were significant more single mothers in AA genotype group than in the PP/PA group (p=0.042).

243 Table 1 Characteristics of study population with TAS2R38 genotype and nutritional
244 data at any time point between 12 and 72 months by TAS2R38 genotype
245 (n=691)

		PP/PA	AA	Total
Country	Germany n (%)	76 (17.1)	47 (19.0)	123 (17.8)
	Belgium n (%)	55 (12.4)	31 (12.6)	86 (12.4)
	Italy n (%)	86 (19.4)	43 (17.4)	129 (18.7)
	Poland n (%)	95 (21.4)	63 (25.5)	158 (22.9)
	Spain n (%)	132 (29.7)	63 (25.5)	195 (28.2)
Sex	Boys n (%)	226 (50.9)	114 (46.2)	340 (49.2)
	Girls n (%)	218 (49.1)	133 (53.8)	351 (50.8)
Maternal educational level	Low n (%)	99 (22.3)	49 (19.8)	148 (21.4)
	Intermediate n (%)	220 (49.5)	121 (49.0)	341 (49.3)
	High n (%)	125 (28.2)	75 (30.4)	200 (28.9)
	Unknown (%)	0 (0.0)	2 (0.8)	2 (0.3)
Maternal age at child's birth (y)	<28 n (%)	127 (28.6)	74 (30.0)	201 (29.1)
	28 - < 33 n (%)	175 (39.4)	98 (39.7)	273 (39.5)
	33 - 44 n (%)	142 (32.0)	74 (30.0)	216 (31.3)
	Unknown n (%)	0 (0.0)	1 (0.4)	1 (0.1)
Type of formula	Low protein n (%)	155 (34.9)	96 (38.9)	251 (36.3)
	High protein n (%)	166 (37.4)	76 (30.8)	242 (35.0)
	Breastfed n (%)	123 (27.7)	75 (30.4)	198 (28.7)
Mother's BMI pre-pregnancy (kg/m²)	< 25 n (%)	303 (68.2)	173 (70.0)	476 (68.9)
	25 - <30 n (%)	102 (23.0)	49 (19.8)	151 (21.9)
	≥ 30 n (%)	30 (6.8)	19 (7.7)	49 (7.1)
	Unknown n (%)	9 (2.0)	6 (2.4)	15 (2.2)
Single mother	Yes n (%)	13 (2.9)	15 (6.1)	28 (4.1)
	No n (%)	431 (97.1)	230 (93.1)	661 (95.7)
	Unknown n (%)	0 (0.0)	2 (0.8)	2 (0.3)
Child's birth order	1st child n (%)	242 (54.5)	150 (60.7)	392 (56.7)
	2nd child n (%)	160 (36.0)	74 (30.0)	234 (33.9)
	≥ 3rd child n (%)	41 (9.2)	23 (9.3)	64 (9.3)
	Unknown n (%)	1 (0.2)	0 (0.0)	1 (0.1)
Total		**444 (64.3)**	**247 (35.7)**	**691 (100)**

Food categorization

In total 4850 different food items were consumed. 1653 (34.1%) were categorized as sweet tasting food items, 927 (19.1%) with low energy density and 726 (15.0%) with high energy density. In table 2 the main food groups containing sweet food items are displayed. While fruit and fruit products, sweetened dairy products, desserts and beverages are mainly in the low energy density group, pastries, sweetened cereals and sweets mainly have a high energy density.

Table 2: Number of food items from relevant food groups contained in sweet food items with either low (<200 kcal/100g) or high (≥200 kcal/100g) energy density n (% of total sweet food items)

	Food items n	**Sweet food items n**	**Sweet food items with low energy density n (%)**	**Sweet food items with high energy density n (%)**
Fruit and fruit products	448	433	382 (88.2)	51 (11.8)
Pastry	298	298	15 (5.0)	283 (95.0)
Sweetened cereals	223	223	67 (30.0)	156 (70.0)
Beverages	241	186	182 (97.8)	4 (2.2)
Sweets	185	185	32 (17.3)	153 (82.7)
Sweet dairy products	133	133	132 (99.2)	1 (0.8)
Desserts	99	96	81 (84.4)	15 (15.6)
Sweet main dishes	59	57	34 (59.6)	23 (40.4)
Instant cacao powder	19	19	1 (5.2)	18 (94.8)
Sugar, honey, syrup	16	16	0 (0.0)	16 (100)
Others	3129	7	6 (85.7)	1 (14.3)

Intake of energy, macronutrients, sugar and sweet food

Table 3 shows the average daily intake of total energy, macronutrients, sugar and energy from sweet tasting foods (with high or low energy density) as well as BMI z-scores. Total sugar intake increased from 67 g/d (30 % of total energy intake [E%]) to 85 g/d (24 E%) and total intakes of sweet tasting foods increased from an average of 1110 kJ/d (30 E%) at 12 months to 2054 kJ/d (33 E%) at 72 months. Total energy, macronutrient and sugar intake, energy intake from single food groups as well as BMI z-score was differed not according to TAS2R38 genotype, but energy intake from sweet tasting foods was significantly higher in children with PP/PA genotype ($p=0.009$).

The composition of consumed sweet tasting foods differed between genotypes: While children with PP/PA genotype have a significant higher energy intake from sweet products with high energy density (on average 56 kJ/d; 95 % CI 15 to 98 kJ/d; $p=0.007$), energy intake from sweet products with low energy density varies not significantly by TAS2R38 genotype ($p=0.200$).

Energy and sugar intake varied considerably between countries over the whole study period. While Spain and Poland had the highest energy intakes, sugar intake was much higher in Poland and much lower in Italy compared to all other countries. Energy intake was highest in Spain (4001 kJ/d) at 12 months and in Poland (6408 kJ/d) at 72 months of age. Sugar intake at 12 months of age ranged from 52 g/d in Italy to 76 g/d in Spain and from 76 g/d in Italy to 95 g/d in Poland at 72 months of age.

Table 3: Means (SD) of intake of energy (kJ), macronutrients (g/d), sugar (g/d and [E%]) and energy intake from relevant food groups (kJ/d and [E%]) and BMI z-score by TAS2R38 genotype over time

Nutrient intake[1]	TAS2R38 genotype	Age (months) Number of participants 12 n=574	24 n=525	36 n=415	48 n=405	60 n=355	72 n=382	p-value[3]
Energy	PP/PA	3703 (750)	4616 (965)	5121 (992)	5537 (1016)	5840 (1064)	6155 (1042)	0.902
	AA	3671 (701)	4736 (1034)	5150 (989)	5497 (995)	5724 (1070)	6120 (960)	
	Total	3696 (732)	4658 (991)	5138 (990)	5526 (1007)	5808 (1065)	6146 (1010)	
Carbohydrates	PP/PA	116 (27)	137 (34)	152 (36)	164 (34)	174 (41)	184 (39)	0.309
	AA	116 (26)	139 (34)	153 (34)	162 (34)	174 (38)	182 (35)	
	Total	116 (27)	138 (34)	153 (35)	164 (34)	175 (40)	184 (38)	
Protein	PP/PA	33 (10)	44 (12)	47 (12)	50 (13)	52 (13)	54 (13)	0.086
	AA	32(10)	46 (13)	48 (11)	50 (12)	50 (13)	55 (12)	
	Total	32 (10)	45 (13)	47 (12)	50 (12)	52 (13)	55 (13)	
Fat	PP/PA	32 (8)	42 (12)	48 (13)	52 (14)	55 (14)	58 (14)	0.704
	AA	32 (8)	43 (13)	47 (12)	52 (14)	53 (13)	57 (14)	
	Total	32 (8)	42 (12)	48 (13)	52 (14)	54 (13)	58 (14)	
Sugar[2]	PP/PA	67 (20) [30]	72 (24) [26]	77 (25) [25]	80 (24) [24]	83 (27) [24]	85 (26) [23]	0.878
	AA	68 (20) [31]	73 (23) [26]	79 (23) [26]	79 (23) [24]	83 (26) [24]	85 (26) [23]	
	Total	67 (20) [30]	72 (28) [26]	78 (24) [26]	80 (24) [25]	84 (27) [24]	85 (26) [24]	
Energy from sweet tasting foods (E%)	PP/PA	1134 (560) [31]	1680 (833) [36]	1788 (745) [35]	1907 (749) [34]	2016 (824) [35]	2047 (819) [33]	0.009
	AA	1069 (524) [29]	1605 (733) [34]	1783 (775) [35]	1816 (756) [33]	1995 (796) [35]	2061 (790) [34]	
	Total	1110 (548) [30]	1653 (799) [35]	1785 (755) [35]	1875 (752) [34]	2009 (813) [34]	2054 (808) [33]	
Energy from sweet products with low energy density (E%)	PP/PA	712 (433) [19]	974 (667) [21]	945 (548) [18]	942 (482) [18]	1011 (580) [17]	1009 (541) [16]	0.200
	AA	701 (406) [19]	966 (640) [20]	995 (528) [19]	906 (470) [16]	970 (543) [17]	981 (496) [16]	
	Total	708 (423) [19]	971 (657) [21]	962 (541) [19]	929 (477) [17]	997 (567) [17]	999 (525) [16]	
Energy from sweet products with high energy density (E%)	PP/PA	422 (362) [11]	707 (485) [15]	843 (514) [16]	966 (576) [17]	1005 (546) [17]	1039 (572) [17]	0.007
	AA	369 (320) [10]	639 (404) [13]	788 (516) [15]	910 (518) [17]	1024 (550) [18]	1081 (575) [18]	
	Total	403 (348) [11]	682 (458) [15]	824 (515) [16]	946 (556) [17]	1012 (547) [18]	1054 (572) [17]	
		12 n=568	**24 n=515**	**36 n=400**	**48 n=394**	**60 n=349**	**72 n=377**	
BMI for age z-score	PP/PA	0.38 (0.95)	0.28 (0.95)	0.37 (0.92)	0.37 (0.98)	0.41 (1.06)	0.42 (1.23)	0.170
	AA	0.30 (1.05)	0.24 (0.96)	0.24 (1.11)	0.32 (0.98)	0.24 (1.02)	0.22 (1.2)	
	Total	0.35 (0.99)	0.26 (0.96)	0.32 (0.99)	0.35 (0.98)	0.35 (1.05)	0.35 (1.21)	

All values are Means (SD)

[1]Dietary intakes measured via three-day dietary records

[2]Mono- and disaccharides (g/d)

[3]Differences in values were assessed via mixed models with random intercept and slope including quadratic and cubic age terms all adjusted for country and gender; additionally adjusted for energy intake in models of macronutrients and sugar and for type of formula in model of BMI for age z-score.

Energy intake from sweet tasting food

Fixed effects of TAS2R38 genotype on energy intake from sweet tasting foods adjusted for country and gender from a mixed model are displayed in Table 4. Children with PP or PA genotype had a significantly higher energy intake from sweet tasting foods than children with AA genotype (difference on average 83 kJ/d; 95% CI 21 to 146; p=0.009). Individual energy intake from sweet tasting food varied considerably. (Fig. 1, table 3 and 4)

Additional adjustment for single mother status, mother's education attainment, marital status, maternal age at birth, birth order or maternal smoking during pregnancy did not attenuate any genotype effect estimates. In the subgroup analysis with further adjustment for BMI and energy misreporting the association of TAS2R38 genotype with energy intake from sweet tasting food was somewhat strengthened, especially after including the misreporting status (+99 kJ/d; 95% CI 35 to 163; p=0.002) in comparison to results in the same subgroup without adjustment (+91 kJ/d; 95% CI 24 to 157; p=0.008).

Girls had a lower energy intake from sweet tasting foods between 12 and 72 month (-128 kJ/d; 95% CI -188 to -68; p<0.001). Intake of sweet tasting food also differed between the participating study countries. Polish children had the highest energy intake per day from sweet tasting foods (+387 kJ/d compared to baseline Spain; 95% CI 302 to 472; p<0.001), while the intake was lowest in Italy (-511 kJ/d compared to baseline Spain; 95% CI -597 to -424; p<0.001). The estimated mean energy intake from sweet food items at 12 months of age after adjustment (=constant term in mixed model) was 1333 kJ/d (95 % CI 1212 to 1455; p<0.001). The estimation of the influence of age on energy intake from sweet food items includes linear terms ('age'; per month +53 kJ/d), quadratic terms ('age^2'; per month -1 kJ/d) and cubic terms ('age^3'; per month 0 kJ/d).

316 Table 4: Fixed and random effects on energy intake from sweet tasting foods from a
317 mixed model with random intercept and slope including fixed cubic age terms (3127
318 observations)
319

Fixed effects		
Variable	**Estimates (kJ) (95% CI)**	**p value**
Genotype PP/PA[1]	83 (21 to 146)	0.009
Female[2]	-128 (-188 to -68)	<0.001
Country[3]		
Germany	-182 (-274 to -90)	<0.001
Belgium	-178 (-282 to -73)	0.001
Italy	-511 (-597 to -424)	<0.001
Poland	387 (302 to 472)	<0.001
Age	53 (46 to 60)	<0.001
Age^2	-1 (-2 to -1)	<0.001
Age^3	0 (0 to 0)	<0.001
Constant term	1333 (1212 to 1455)	<0.001
Random effects		
Variance (intercept)	103 (78 to 135)	
Variance (constant term)	87496 (65668 to 116579)	
Covariance (intercept, constant term)	-804 (-1501 to -107)	

320 [1] baseline: genotype PP/PA
321 [2] baseline: male
322 [3] baseline: Spain
323

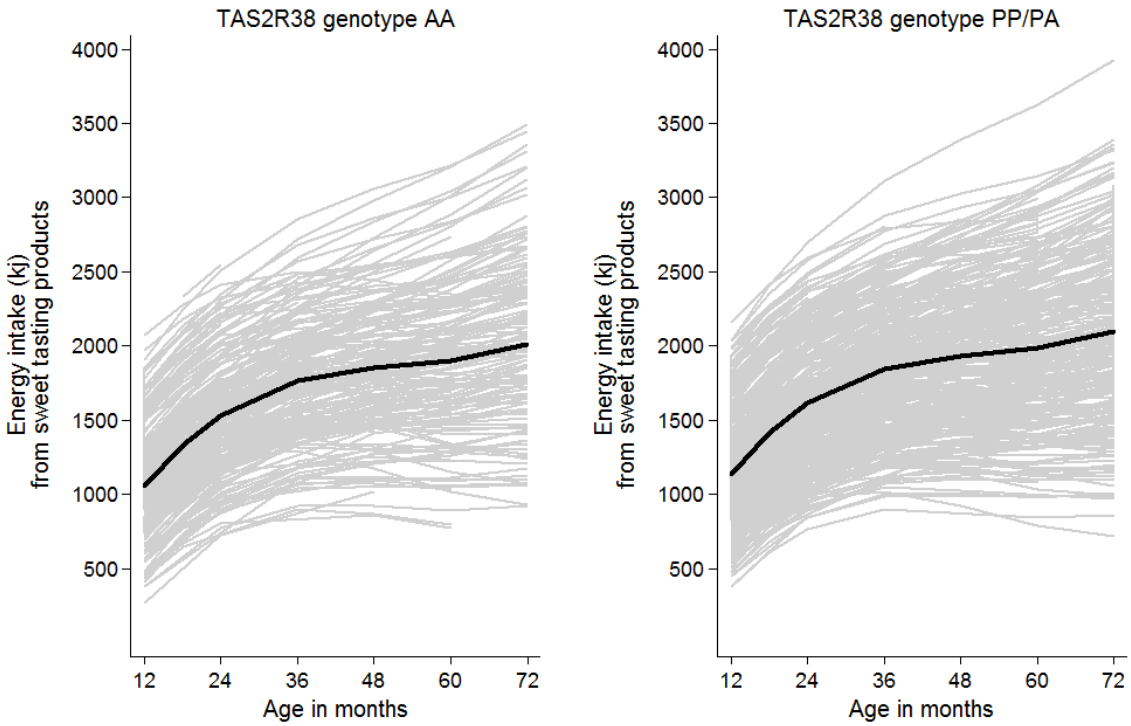

Fig. 1 Subject-specific and population averaged development of energy intake from sweet tasting products adjusted for country and gender by TAS2R38 genotype from 12 up to the age of 72 months (results of a mixed model with random intercept and slope with quadratic and cubic age terms)

Discussion

The PP/PA genotype of the bitter taste receptor gene TAS2R38 was associated with a higher intake of (energy dense) sweet tasting foods in children from five European countries. Further associated factors of sweet food intake were gender and country. The intake of macronutrients and sugar as well as BMI z-score and single food groups was not significantly associated with the TAS2R38 genotype.

Association of TAS2R38 genotype with intake of (energy dense) sweet food

In former studies the taste receptor TAS2R38 was reported to influence the preference for sweet taste and intake of sweet tasting food in children's food (Mennella, Pepino & Reed, 2005; Keller & Tepper, 2004; Joseph, Reed & Mennella, 2016). We could confirm that the consumption of sweet foods is associated with TAS2R38 genotype. Several studies linked sugar intake or sweet liking with PROP phenotype, which is explained for 55-85 % by TAS2R38 genotype: Keller and Tepper (2004) and Keller et al. (2014) reported a higher percentage of daily energy intake from sugars in tasters compared to non-taster children aged 4 to 5 years: At a palatable test-meal, taster children consumed more energy from the food group "sweets" than non-taster children, but this was not seen in another study of Keller et al. (2010). Two studies of Keller et al. (2010; 2014) found no effects of TAS2R38 genotype on food selection, while in a study of Joseph, Reed & Mennella (2016) children with two bitter sensitive alleles in the TAS2R38 gene reported a higher sugar intake than children with less sensitive alleles.

We further explored the difference of the effect of the TAS2R38 genotype on sugar intake - no effect - and energy intake from sweet products by comparing the effect on energy from sweet tasting products with high and low energy density. We observed that children with genotype PP or PA have a significantly higher energy intake from sweet products with a high energy density (≥ 200 kcal/100g), but not from sweet

products with low energy density. The higher energy intake from sweet tasting foods of PP/PA children can be explained by the added energy intake provided by fat, other carbohydrates and protein contained in higher amounts in energy dense sweet products. In that food group many products generally identified as unhealthy like sweets or pastry products are contained, hence children with TAS2R38 genotype PP or PA tend to have a less balanced and healthy diet. The overall effect of the observed difference is low if purely the energy aspect is considered. However, our observation points also to differences in the dietary quality, which is known to influence lifelong health aside from high energy intake and obesity. As we did not find differences in total energy intake by genotype, the differences consist of a shift in-between food groups, which could influence dietary quality via intake of micronutrients, fiber or phytochemicals. In a report of the United States Department of Agriculture it is summarized that dietary patterns characterized by lower consumption of sugar-sweetened foods and beverages and sweets are associated with lower risk for cardiovascular diseases, type 2 diabetes and some forms of cancer. (United States Department of Agriculture [USDA], 2015)

Association of gender and country with sweet food intake

In accordance with our findings, Bjelland et al. (2013) also reported that boys have a significantly higher sweet intake namely of sweetened beverages at 18 months of age than girls. Similar in a US study in high school students: boys had a greater odds ratio for high intake of sugar sweetened beverages than girls (Park, Sherry & Blanck, 2012).

Differences in consumption of sweet tasting foods across European countries were also reported in the HELENA study in adolescents that found the highest energy intake from sweet tasting beverages in Germany (1792 kJ/d) and the lowest in Italy (834 kJ/d). Spanish and Belgian adolescents had an intermediate intake of sweet

beverages (Duffey et al., 2012). Previous analyses in the CHOP cohort showed that the consumption of energy providing liquids in infancy was highest in Poland (Schiess et al. 2010). Traditionally, Poland has a high consumption both of meat dishes as well as cakes and pastries, which appear to contribute to the high energy intake from sweet tasting foods in Polish children.

Association of TAS2R38 genotype with BMI

Negri et al. (2012) found an association of taster status (determined by threshold tests) with BMI in boys but not girls: among the obese boys or girls there was no supertaster, but 32% of the normal weights were supertasters. Inoue et al. (2013) reported that homozygote carriers of the non-taster variant are taller and heavier, but that BMI does not differ between TAS2R38 genotypes. We could not find an effect of TAS2R38 genotype on BMI z-score just as in a German adult population where no associations between TAS2R38 genotype and BMI were found either (Sausenthaler, Rzehak, Wichmann & Heinrich, 2009). Baranowski et al. (2010) examined the BMI of children in the age of 9-10 and 17-18 years and found that PROP supertasters had the largest BMI percentile and z-score in children with the highest socioeconomic status, while in children with lower socioeconomic status the influence of PROP taster status is covered by other factors. Similar results were found by Burd, Senerat, Chambers & Keller (2013), who examined the interaction of children's BMI, the food environment and PROP taster status: Non-taster children from unhealthy food environments had higher BMI z-scores than all other groups. Obviously, taster status is only one factor influencing children's BMI and interacts with socioeconomic factors.

Association of TAS2R38 genotype with other variables of food intake

While a Japanese study on female college students aged 19 to 21 years found a higher intake of total energy and carbohydrates in carriers of homozygote non-taster variant (Inoue et al., 2013), O'Brien, Feeney, Scannell, Markey & Gibney (2013)

found no association either between TAS2R38 genotype nor PROP sensitivity and energy intake, nutrient intake or food group selection in Irish children aged 7 to 13 years. Similarly, we did not find a significant effect of TAS2R38 genotype on total energy intake, nor on the total intake of sugar and other nutrients or single food groups. In elderly women in Brazil, no associations of the rs713598 polymorphism of the TAS2R38 gene were found with any food group, except for bitter tasting vegetables (Colares-Bento et al., 2012). The lack of associations between TAS2R38 genotype and the intake of single food groups may also be due to our tool for nutritional assessment (three-day dietary records) which is appropriate for evaluation of macronutrients and large food groups but less appropriate for evaluation of rarely consumed food groups (Magarey et al., 2011).

Strengths and limitations

An obvious strength of our investigation is the study of children from five countries across Europe; thus our results are indicative of effects across populations. Furthermore, we assessed dietary intake via detailed three-day dietary records which is considered to be the most precise method of dietary intake assessment in young children. We used a standardized approach with quality assurance to data collection and evaluation that is expected to reduce bias and errors.

Although there are three SNPs in the TAS2R38 gene, which are associated with bitter sensitivity, it is considered sufficient to genotype the first one because they are in strong linkage disequilibrium (Kim et al., 2003).

Though studies relating TAS2R38 genotypes with PROP/PTC phenotypes find an intermediate sensitivity to the bitter compounds in heterozygous individuals (Behrens, Gunn, Ramos, Meyerhof & Wooding, 2013) with a wide range of sensitivity to PROP and similar bitter compounds (Lipchock, Mennella, Spielman & Reed, 2013), studies that try to find relations between TAS2R38 genotypes and food intakes or food

preferences mostly find the same relations for homozygous and heterozygous individuals (Mennella, Pepino & Reed, 2005; Colares-Bento et al., 2012). Furthermore, heterozygous children seem to be more bitter sensitive than heterozygous adults (Mennella, Pepino & Reed, 2005, Negri et al. 2012). For this reason, we decided to pool homozygotes (genotype PP) and heterozygotes (genotype PA) together in one group. The distribution of TAS2R38 genotype differed slightly in all five study countries, but is comparable to results from other studies in Caucasian populations (Kim et al., 2003).

We did not test the bitter sensitivity via PROP solution threshold tests, as this was done in several similar studies. The TAS2R38 genotypes explains up to 85 % of the variation in PROP sensitivity depending from the examined population (Kim et al., 2003). So it might be a limitation that there could be children in the PP/PA genotype group, which would be classified as non-tasters by phenotype and vice versa. In the study of Keller, K.L., Reid, A., MacDougall, M.C., Cassano, H., Song, J.L., Deng, L., et al. (2010) in 4-6 years old children in 75.8 % of the children genotype predicted PROP phenotype, but in 24.4 % not. Other factors influencing PROP sensitivity are not well defined: e.g. Timpson et al (2007) found no association with social factors or depression.

The German food database BLS was selected as basis for the calculation of nutrient intakes from the three-day dietary records in all participating countries as it is the largest food database available in the participating countries. For typical local products or any other products not contained in the BLS the dieticians transferring the dietary records had the possibility to add new food items to the database. Thus, the database we used for this study contained pure food items (as vegetables, fruits or meat) and also valid nutrient data for local products and recipes.

The number of participants decreased from 574 at the age of 12 months to 382 at the age of 72 months, partly due to the time consuming and demanding task of completing the three-day dietary records, in addition to the request to complete multiple other questionnaires as part of the large multicentre CHOP trial. Children who stayed in the study came generally from families with a higher socio-economic status. Thus, some bias might be possible despite the fact that we did not find any effect modification by age.

We did not investigate food groups with the affected bitter compounds but sweet tasting foods. We hypothesized that children that are more bitter sensitive consume more sugar and sweet tasting food to compensate for the bitter taste perception. As expected, we can only find a modest effect of TAS2R38 bitter receptor variants on food selection and intake: First of all only part of the variation in the sensitivity to the bitter glucosinolates is explained by the analysed genetic variants (Kim et al., 2003; Kim & Drayna, 2005), secondly, the TAS2R38 bitter receptor is only one of 25 bitter receptors (TAS2Rs) (Hayes, Feeney & Allen, 2013), and thirdly, individual differences in perception of taste and odours have also been associated with polymorphisms in other bitter receptor genes and in genes involved in umami and sweet tastes or odors perception (Nothlings, Murphy, Wilkens, Henderson & Kolonel, 2007). Therefore, TAS2R38 is expected to explain only a small part of total genetic taste variance.

The detected effects of genetic variants of the TAS2R38 gene on the intake of sweet foods and particularly on sweet products, generally considered as unhealthy may affect long-term health outcomes such as obesity, adiposity and other nutrition related diseases, which deserve further studies.

Conflict of interest statement

The authors declare no conflict of interest.

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Contribution of authors

IP collected, analysed and interpreted data, searched for relevant literature and wrote the paper.

RC, AS, EV, AX collected genetic and dietary data in their respective countries.

VG analysed and interpreted data.

ER performed the genetic analysis.

PR helped analysing data.

BK designed the study.

All authors had final approval of the submitted and published version of the paper.

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