An exploratory study on Smith Magenis syndrome:

differences in weight and lipid profiles between patients with a 17p11.2 deletion and patients with a RAI1 gene mutation

Sterre Tromp[1]

Marie-José van den Boogaard[1]

Michiel Houben[1]

Esther de Rooij[2]

Wiebe Braam[2]

Agnies van Eeghen[2]

Erik Boot[2-4]

1. *Universitair Medisch Centrum Utrecht, Utrecht*
2. *'s Heeren Loo Zorggroep, Amersfoort*
3. *Dalglish Family 22q Clinic, Toronto, Ontario, Canada*
4. *Afdeling Psychiatrie en Neuropsychologie, Maastricht Universitair Medisch Centrum+, Maastricht*

Dit onderzoek werd uitgevoerd bij het expertisecentrum genetische syndromen van 's Heeren Loo, in het kader van een wetenschapsstage/keuzeonderwijs bij de afdeling Klinische Genetica van het Universitair Medisch Centrum Utrecht.

Samenvatting

Achtergrond: Het Smith Magenis syndroom (SMS) is een genetische ontwikkelingsstoornis die wordt veroorzaakt door een deletie van 17p11.2 of een mutatie in het RAI1 gen dat in de 17p11.2 regio ligt. SMS wordt gekarakteriseerd door een verstandelijke beperking, slaap-, gedrags- en somatische problemen zoals overgewicht en stoornissen in het lipidenspectrum. Genotype-fenotype relaties zijn in slechts enkele studies beschreven. Het doel van deze studie was om meer inzicht te krijgen op het gebied van overgewicht en stoornissen in het lipidenspectrum, en de verschillen tussen patiënten met een deletie en patiënten met een mutatie.

Methode: Het betreft een retrospectief dossieronderzoek van 38 patiënten met SMS (27 met een 17p11.2 deletie en 11 met een RAI1 mutatie) in de leeftijd van 2-37 jaar (10 ≥18 jaar bij laatste bezoek). Er waren geen groepsverschillen in leeftijd (p=0.25) of geslacht (p=0.47). Gegevens over genotype, body mass index (BMI) en lipidenprofiel waren op systematische wijze verzameld uit de dossiers.

Resultaten: Overgewicht werd gevonden in 30% van de patiënten met een 17p11.2 deletie en in 73% van de patiënten met een RAI1 mutatie (p=0.03). Na correctie voor leeftijd en geslacht hadden patiënten met een RAI1 mutatie een grotere kans op overgewicht dan patiënten met een 17p11.2 deletie (OR=5.75, 95% CI=1.17-28.11). De mediane BMI in volwassenen was 24.6 kg/m2 (IQR=23.7-32.6). Gegevens over het lipidenspectrum waren voor slechts een deel van het cohort beschikbaar (n=24); afwijkingen werden alleen gevonden in patiënten met een 17p11.2 deletie.

Conclusie: Onze resultaten sluiten aan bij de eerder gerapporteerde verschillen tussen patiënten met een 17p11.2 deletie en patiënten met een RAI1 mutatie, waarbij patiënten met een mutatie meer kans hebben op overgewicht, terwijl patiënten met een deletie meer kans hebben op afwijkingen in het lipidenspectrum. Verdere studies in grotere groepen zijn nodig om andere genotype-fenotype relaties te onderzoeken.

Abstract

Background: Smith Magenis syndrome (SMS) is a genetic neurodevelopmental disorder caused by an interstitial deletion of 17p11.2 or a mutation in the RAI1 gene,

located in the 17p11.2 region. SMS is characterized by intellectual disability, sleep disturbances, behavioural problems and several somatic conditions including obesity and lipid disorders. To date, few studies have addressed genotype-phenotype associations. The aim of this study was to enhance the knowledge on the relationship between body weight, lipid profiles, and the cause of SMS (deletion vs. RAI1 mutation).

Methods: A retrospective chart study was conducted of 38 individuals with SMS (27 with a 17p11.2 deletion and 11 with a RAI1 mutation) aged 2-37 years (10 aged ≥18 years at last assessment) There were no between-group differences in age (p=0.25) or sex (p=0.47). Data concerning genotype, body mass index (BMI) and lipid profiles were collected during regular visits and extracted by systematic chart reviews.

Results: Overweight was present in 30% of patients with a deletion and in 73% of patients with a RAI1 mutation (p=0.03). After correction for age and sex, individuals carrying a RAI1 mutation were more likely to have overweight compared to those carrying a 17p11.2 deletion (OR=5.75, 95% CI=1.17-28.11). The median BMI in adults was 24.6 kg/m2 (IQR=23.7-32.6). Abnormal lipid profiles, available for a subset of the cohort (n=24), were only found in patients with an interstitial deletion.

Conclusion: Our results add to the previously reported, and seemingly conflicting, findings suggesting a higher risk on overweight in those with a RAI1 mutation, and a higher risk on lipid profile abnormalities in those with a 17p11.2 deletion. Further research in larger sample sizes is required to explore additional genotype-phenotype associations.

Introduction

Smith Magenis syndrome (SMS) is a genetic neurodevelopmental disorder characterized by intellectual disability, sleep disturbances and behavioural problems.[1,6] SMS is caused by an interstitial deletion within chromosome 17p11.2 (90%) or a mutation of the retinoic acid-induced 1 gene (RAI1) in this region (10%),[1,3,7-8] and has an estimated occurrence of 1:15000-25000 births.1,9 Phenotypically, there is a significant overlap among individuals with the deletion and individuals with a RAI1 mutation, but there appear to be some differences as well.[2-3,10,12]

Common features including overweight, hypercholesterolemia, and other endocrinological problems, might be related to circadian rhythm disruptions due to haploinsufficiency of RAI1.[4,13-14] Genotype-phenotype associations have been described in relatively small cohorts and suggest that overweight is more prominent in individuals with a RAI1 mutation, compared to those with a 17p11.2 deletion.[3,12] To further explore this genotype-phenotype association, we evaluated overweight and lipid profiles in a Dutch cohort of 38 individuals with SMS.

Material and methods

Study design and setting

A retrospective chart review was conducted of individuals with a molecularly confirmed diagnosis of SMS who visited the national SMS outpatient clinic at 's Heeren Loo, between January 2003 and January 2020. The study was not subject to the Medical Research Involving Human Subjects Act; a waiver for formal approval was obtained from the Institutional Review Board of Amsterdam UMC, The Netherlands.

Participants

Thirty-eight individuals (27, 71% with a 17p11.2 deletion and 11, 29% with a RAI1 mutation) aged 2-37 years were included in the study (table 1). There were no statistically significant differences in age and sex between those with a 17p11.2 deletion and RAI1 mutation. All cases with information on inheritance status had a de novo 17p11.2 deletion/RAI1 mutation (n=29 of 38 [76%]).

Clinical characteristics

Clinical data concerning genotype, body mass index (BMI), and lipid profiles (total cholesterol, HDL, LDL and triglyceride) were collected from medical charts, intake forms completed by parents, and laboratory assessments. Individuals were categorized into normal (underweight and normal) vs overweight (overweight and obese) groups, and normal vs abnormal lipid groups. Adults (≥18 years) with a BMI ≥25 kg/m2 were considered overweight.[15] Children were considered overweight according to standard age- and gender-dependent criteria.[16] To assess normality on total cholesterol, LDL, HDL and triglyceride levels, we used local criteria for adults,[17] and in children we used standard age specific reference values.[18]

Statistical analyses

Statistical analyses were performed using IBM SPSS statistics 25 (Inc., Chicago, IL). We used the Kolmogorov-Smirnov test to assess normality of the continuous variables. Since most variables were not normally distributed, we decided to use non-parametric tests for all continuous variables. Multivariate analysis was performed to assess the independent factors that may be associated with overweight (genotype, sex, age). All reported p-values were two-tailed with a significance level of 0.05. Missing values on a particular outcome were excluded for that analysis.

Table 1: Demographics of 38 individuals with Smith Magenis syndrome.

	Total N=38		**17p11.2 deletion N=27**		**RAI1 mutation N=11**		**Statistics**
	Median	IQR	Median	IQR	Median	IQR	*p* [a]
Age at last assessment, y	13.0	8.0-18.8	12.0	8.0-18.0	15.0	9.0-27.0	0.25
Age at diagnosis, y	4.0	2.0-10.5	4.0	1.0-6.0	10.0	4.0-21.0	**0.02**
	Count	%	Count	%	Count	%	*p* [b]
Males	22	58	17	63	5	46	0.47
Adults	10	26	7	26	3	27	1.00
Use of melatonin	22	58	14	52	8	73	0.30
Use of antipsychotics	11	29	8	30	3	27	1.00
Use of stimulants	6	16	5	19	1	9	0.65

Bold font indicates statistical significance. [a] Mann-Whitney U test. [b] Fisher's exact test. IQR = interquartile range, y = years.

Table 2: Overweight and lipid profiles in individuals with Smith Magenis syndrome.

	Total	**%**	**17p11.2 deletion**	**%**	**RAI1 mutation**	**%**	**Odds ratio (95% CI)**	***p*** [a]
Overweight	16/38	42	8/27	30	8/11	73	6.33 (1.33 - 30.23)	**0.03**
Increased total cholesterol	0/23	0	0/18	0	0/5	0	-	-
Increased LDL	2/23	9	2/19	11	0/4	0	-	1.00
Increased triglyceride	5/21	24	5/17	29	0/4	0	-	0.53
Decreased HDL	3/24	13	3/19	16	0/5	0	-	1.00

Bold font indicates statistical significance. All data are based on last assessment available. [a] Mann-Whitney U test. 95% CI=95% confidence interval.

Results

Data on nutritional status and lipid profiles are presented in table 2. Only overweight-rates differed statistically significant between individuals with a 17p11.2 deletion (8/27, 30%) and those with a RAI1 mutation (8/11, 73%, p=0.03). The median BMI in adults was 24.6 kg/m2 (interquartile range, IQR=23.7-32.6).

There was a strong positive relationship between age and BMI in both genotype groups (figure 1). No difference was observed between patients who did and those who did not use antipsychotics.

The multivariable regression model assessing the independent contribution of different variables on BMI showed that genotype was associated (OR=5.75, confidence interval, CI=1.17-28.11; table 3 - next page). This indicates that, corrected for the factors age and sex, individuals carrying a RAI1 mutation are at increased risk to develop overweight, compared to those carrying a 17p11.2 deletion.

Only five individuals (45%) with a RAI1 mutation had data on their lipid profile. None of them had abnormal lipid or triglyceride levels.

Table 3: Independent factors associated with overweight in 38 individuals with Smith Magenis syndrome[a].

	Odds ratio	95% CI	p-value
Genotype (RAI1 mutation)	5.75	1.17 - 28.11	**0.03**
Sex (Female)	1.31	0.30 - 5.78	0.65
Age	1.02	0.94 - 1.11	0.65
Full model	0.30	-	0.08

Bold font indicates statistical significance. All data are based on last assessment available. [a] Multivariate analysis assessing the impact of independent factors on the likelihood of overweight in Smith Magenis syndrome.

Table 4: Studies on overweight in Smith Magenis syndrome.

Authors	Year	N	Age, y	Age range, y	Overweight, %
This study	2020	17p del: 27 RAI1: 11	M: 12 M: 15	0-37 5-36	30 73
Alaimo et al.	2015	[a] 99	$\bar{x}$: 14	1-51	36
Burns et al.	2010	[a] 38	$\bar{x}$: 14	3-51	39
Vilboux et al.	2011	17p del: 49 RAI1: 5	M: 15 M:14	1-49 5-20	57 80
Edelman et al.	2007	17p del: 31 RAI1: 9	M: 8 M: 15	[a] 0-72	13 67
Smith et al.	2002	[a] 49	M: 6	0-17	24

[a] No distinction was made between those with a 17p11.2 deletion and those with a RAI1 mutation.
17p del = 17p11.2 deletion. RAI1 = RAI1 gene mutation. $\bar{x}$ = mean. M = median. y = years.

Discussion

In line with previous studies,[3,12,20,22,28] our findings suggest that overweight is common in individuals with SMS, and more prominent in those with a RAI1 mutation (table 4). This supports the current recommendations for individuals with SMS describing that nutritional status should be evaluated regularly, that individuals should be encouraged to stay physically active from young age, and that every individual should be counselled on the risk of developing overweight.[1]

Overweight was found in 44% of our patients, which is slightly higher than the 25% observed in a previous study with data on overweight in 40 individuals with SMS (including 9 with a RAI1 mutation). The individuals in that study were a few years younger on average compared to this study, and weight was parental reported.[3]

Even though BMI increased with advancing age (figure 1 - next page), multivariate analysis did not identify age as a predictive factor for the presence of overweight. This could possibly be explained by age-dependent cut-off values for overweight in children, and the relatively small proportion of adults in the study.

The high prevalence of overweight in SMS is suggested to be a result of a complex feedback loop between RAI1, circadian locomotor output cycles kaput (CLOCK) genes, and brain derived neurotrophic factor (BDNF), which is downregulated in a SMS mouse model (RAI+/- mice), resulting in impaired satiety and hyperphagia.19,20 This would however not explain why those with a RAI1 mutation had overweight more frequently compared to those with a 17p11.2 deletion.[3] One might hypothesize that absence of other genes in the deleted 17p11.2 region may play a role. Alternatively, one could imagine that the difference in overweight rates between the two groups might reflect the difference in level of overall functioning,[21] making those with a RAI1 mutation more sophisticated in food-seeking behaviour. Future studies are needed to further explore this latter possibility, that may, if proven to be true, inform genotype-specific interventions.

We found lower prevalence rates of abnormal lipid profiles (33%) than the 57% in children reported in previous studies.[20] Interestingly, none of the five individuals with

a RAI1 mutation and data on lipid profiles had abnormal lipid levels. This is in line with SMS mouse models.[23-24] Importantly, abnormal lipid profiles in childhood and adolescence are in general associated with atherosclerosis and adverse cardiovascular outcomes.[25] Treatment of abnormal lipid profiles in SMS is usually limited to dietary regimens.[26]

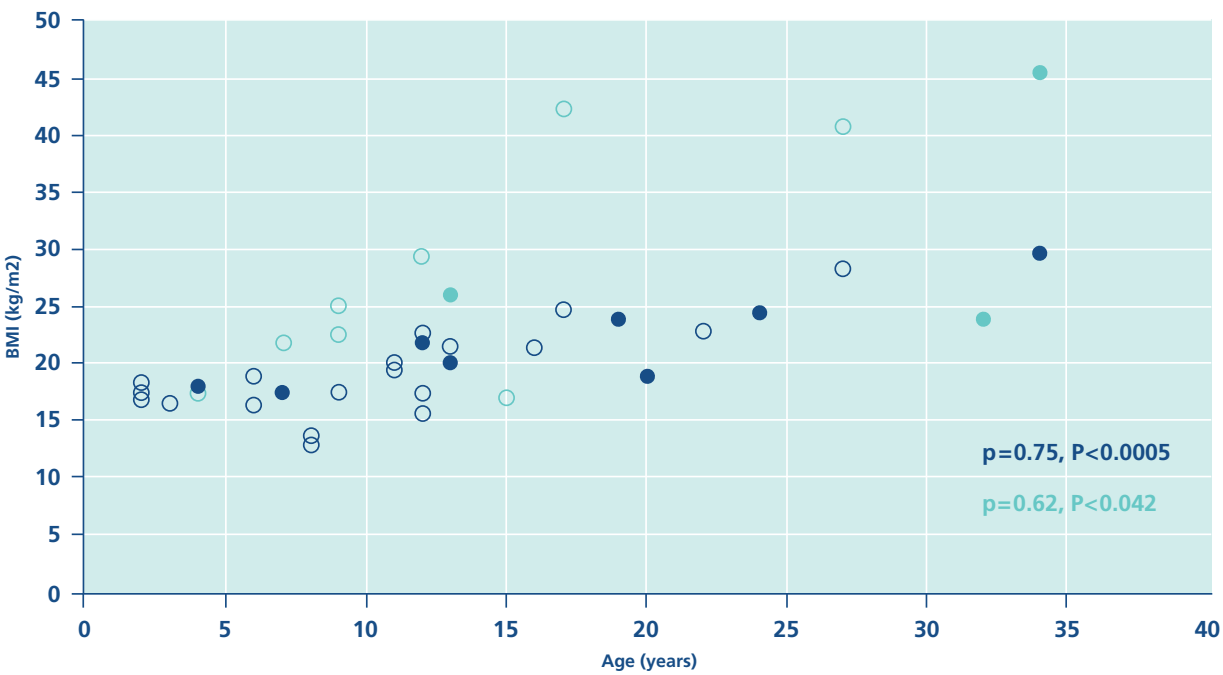
It has been postulated that adverse lipid levels in SMS are a consequence of dysregulation of downstream genes and pathways caused by RAI1 haploinsufficiency, a gene that has an important role in circadian rhythmicity;[19,27] cholesterol biosynthesis and fatty acid metabolism are regulated in a circadian manner.[4,13] Other research suggests that adverse lipid levels might be due to haploinsufficiency of the sterol regulatory element binding transcription factor 1 (SREBF1) gene in the 17p11.2 region.[22-23] Our results would support the second hypothesis, given that abnormal lipid levels were only found in those with a 17p11.2 deletion, also suggesting that annual evaluation of the lipid profile is only necessary in those with a deletion. However, it should be noted that previous studies did not distinguish lipid profiles between carriers of 17p11.2 deletions and RAI1 mutations.[3,20,22]

Strengths of the current study include the relatively large number of individuals with a RAI1 mutation.[3,12,20,22,28]

The study has also some limitations. An important consideration is that, although the age at last assessment did not differ statistically significantly, most individuals with a 17p11.2 deletion were assessed at younger ages compared to those with a RAI1 mutation. Therefore, they had less time to develop overweight. The sample size was too small to assess the true contribution of antipsychotics on weight and lipid profile abnormalities. Also, our cohort, like in the previous studies, is relatively young, with only five patients being older than 25 years. As a result of the retrospective design, some weight data were parent-reported, and there was a considerable amount of missing data on lipid profiles.

In conclusion, the findings of this study support previously reported differences in overweight in SMS, indicating that those with a RAI1 gene mutation have a higher risk of developing overweight, while those with a 17p11.2 deletion have a higher risk on abnormal lipid profiles. Future studies evaluating the underlying mechanisms, and the contribution of antipsychotic drugs, are needed to be able to provide more personalized recommendations for the management of weight and lipid profile abnormalities in SMS. Furthermore, extensive genetic evaluation with novel techniques might reveal other genes and modifiers that explain phenotypic differences between individuals with SMS.

Figure 1: The relationship between age and body mass index in 38 individuals with Smith Magenis syndrome.



Scatterplot of the relationship between age (years) and body mass index (BMI, kg/m2) at last assessment. Dots represent individuals with Smith Magenis syndrome: blue (●) those with a 17p11.2 deletion, turquoise (●) those with a RAI1 mutation, and filled those on antipsychotics. Spearman's rho correlations are shown to the right of the plot.

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