123I-mIBG assessed cardiac sympathetic activity: standardizing towards clinical implementation
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Citation for published version (APA):

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Cardiac sympathetic activity in 22q11.2 deletion syndrome

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ABSTRACT

Aim
22q11.2 Deletion syndrome (22q11.2DS) affects catechol-O-methyl-transferase (COMT), which involves the degradation of norepinephrine (NE). Clinically, adults with 22q11.2DS are at increased risk for sudden unexpected death. Although the causes are likely multifactorial, increased cardiac sympathetic activity with subsequent fatal arrhythmia, due to increased levels of NE, should be considered as a possible mechanism predisposing to this premature death. The purpose of this study was to determine whether cardiac sympathetic activity is increased in 22q11.2DS, both at baseline and following an acute NE depletion with alpha-methyl-para-tyrosine (AMPT).

Materials and methods
Five adults with 22q11.2DS and five age- and sex-matched healthy controls underwent 2 sessions with either AMPT or placebo administration before 123I-mIBG scintigraphy. Heart-to-mediastinum (H/M) ratios were determined from the images 15 minutes (early) and 4 hours (late) after administration of 123I-mIBG and the 123I-mIBG washout (WO) was calculated as an indicator of adrenergic drive.

Results
At baseline there were no significant differences in both early and late H/M ratio between 22q11.2DS and controls. However, there was a significant difference in 123I-mIBG WO between 22q11.2DS and controls (-4.92 ± 2.8 and -10.44 ± 7.2, respectively; p = 0.027), but a “negative” 123I-mIBG WO does not support an increased sympathetic drive. In addition there was a trend towards a higher late H/M ratio after AMPT administration compared to baseline which was more pronounced in 22q11.2DS.

Conclusion
This study for the first time suggests normal cardiac sympathetic activity in adults with 22q11.2DS assessed by 123I-mIBG scintigraphy. Although there is a small difference in adrenergic drive compared to healthy subjects, this most likely does not explain the increased unexpected death rate in the 22q11.2 DS population.
INTRODUCTION

22q11.2 Deletion syndrome (22q11.2DS) is caused by a microdeletion on the long arm of chromosome 22 and affects approximately 1:2000 live births. This genetic condition has a highly variable clinical phenotype with amongst others congenital heart disease (CHD) and psychiatric disorders. The deleted region in 22q11.2DS spans more than 40 genes, one of which is the gene that encodes for catechol-O-methyl-transferase (COMT). This enzyme involves the degradation of catecholamines, including dopamine (DA) and norepinephrine (NE) (Figure 1). People with 22q11.2DS have haploinsufficiency of COMT, resulting in lower enzymatic activity, which may result in abnormal catecholamine levels. Indeed, we showed that in 22q11.2DS subjects urinary DA concentrations are increased and urine and plasma levels of DA metabolites are decreased compared with healthy subjects. In addition, acute monoamine depletion paradigms using alpha-methyl-para-tyrosine (AMPT), a reversible inhibitor of the first and rate-limiting step in the biosynthesis of catecholamines (Figure 1), has been used successfully to assess endogenous brain DA in vivo.

Clinically, individuals with 22q11.2DS who survive childhood have diminished life expectancy and have an increased risk of sudden unexpected death. Although the causes are likely multifactorial, hyperactivity of the cardiac sympathetic system is an important factor for the pathophysiology of fatal arrhythmias including enhance automaticity, triggered automaticity and reentrance. Therefore increased cardiac sympathetic activity, due to increased levels of NE, should be considered as a possible mechanism predisposing to premature death in 22q11.2DS. However, to the best of our knowledge, no data are available whether the cardiac sympathetic system is affected in adults with 22q11.2DS.

NE is a sympathetic neurotransmitter that stimulates the β-adrenoreceptors, which induces positive chronotropic and inotropic effects. Meta-iodobenzylguanidine (mIBG), a NE analog, shares the same presynaptic uptake, storage and release mechanism as NE. Radiolabeling with $^{123}$I allows assessment of presynaptic $^{123}$I-mIBG uptake through the uptake-1 mechanism (i.e. NE transporter). This non-invasive technique has been extensively validated and shown to be of clinical value in many cardiac diseases.

We hypothesized that, due to COMT haploinsufficiency, people with 22q11.2DS are exposed to increased cardiac levels of NE and thereby have an increased cardiac sympathetic activity. In addition, the effect of acute monoamine depletion by AMPT could give additional information about the cardiac NE metabolism. Therefore, the purpose of this study was to determine whether cardiac sympathetic activity assessed with $^{123}$I-mIBG scintigraphy, both at baseline and following an acute depletion challenge with AMPT, is different in adults with 22q11.2DS (without CHD) compared with healthy controls.
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Tyrosine \(\xrightarrow{TH}L\)-DOPA \(\xrightarrow{MAO, COMT}Dopamine \xrightarrow{COMT}Norepinephrine\)

**Figure 1.** Catecholamine metabolism and the negative effect of alpha-methyl-para-tyrosine (AMPT), a reversible inhibitor of tyrosine hydroxylase (TH), on the biosynthesis of catecholamines. MAO, monoamine oxidase; COMT, catechol-O-methyl-transferase; DOPAC, 3,4-dihydroxyphenylacetic acid; 3-MT, 3-methoxytyramine; DHPG, dihydroxyphenylglycine; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol.

**MATERIAL AND METHODS**

**Subjects**
Adults with 22q11.2DS were recruited through the Dutch 22q11.2DS Family Association. For each 22q11.2DS subject, an age- and sex-matched healthy control was included. Inclusion criteria for all subjects were as follows: (1) no current or past psychiatric history, (2) no current or previous exposure to antipsychotics or stimulant medication; (3) no CHD, proven by echocardiography; and for 22q11.2DS subjects (4) a deletion at the 22q11.2 region as determined by fluorescent in-situ hybridization. All subjects provided written informed consent. The study was approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonization–Good Clinical Practice.

**Protocol**
Subjects underwent 2 identical sessions separated by a 2 week interval in which they received either AMPT or placebo. Subjects were randomized and blinded to oral administration of AMPT or placebo. After administration of AMPT or placebo all subjects underwent \(^{131}\text{I}-mIBG\) scintigraphy and blood samples were drawn for analysis of prolactin (PRL) at baseline and after AMPT administration.

**Questionnaires**
To observe for extrapyramidal side-effects of AMPT, all subjects were assessed with the Simpson Angus Scale, Beck Anxiety Inventory and Subjective Well-being under Neuroleptic treatment scale at baseline and 8 h after the initial AMPT dose (\(T_0\) and \(T_8\)).

\(^{131}\text{I}-mIBG\) scintigraphy
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Depletion regimen

The AMPT competition model is a well-accepted paradigm to assess endogenous brain DA. Due to the acute, but reversible, decrease in DA production induced by AMPT, the availability of $^{123}$I-iodobenzamide ($^{123}$I-IBZM) to bind to free striatal DA $D_{2/3}$-receptors is increased. $^{123}$I-IBZM is a selective DA antagonist that binds to dopamine $D_{2/3}$-receptors. Comparing DA $D_{2/3}$-receptor binding at baseline and in the AMPT-induced DA depleted state provides an indirect assessment of endogenous brain DA. This paradigm is now used to assess the cardiac NE. The administration of AMPT induces not only a decrease in production of DA but also of NE (Figure 1). We hypothesized that acute, but reversible decrease in NE production induced by AMPT result in less competition between $^{123}$I-mIBG and NE resulting in increased $^{123}$I-mIBG uptake. Based on our experience in previous studies a total dose of 1500 mg AMPT (Pharmaceutics International Inc., United States of America) was administered over 4 h (Figure 2).

Prolactin

DA is the predominant hypothalamic inhibiting factor of PRL release in humans, and DA $D_3$ receptor stimulation has inhibiting effects on PRL release in the anterior pituitary. $^{16}$ In addition, in sheep NE has inhibiting effects on PRL release. $^{17}$ Previously, PRL levels have been used as a proxy marker of the effectiveness of catecholamine depletion by AMPT. $^{18}$ Therefore, plasma concentrations of PRL were measured at baseline and at 4 and 6 h after the first AMPT administration ($T_0$, $T_4$, and $T_6$, respectively).

$^{123}$I-mIBG scintigraphy acquisition and analysis

To block uptake of free $^{123}$I by the thyroid gland, subjects were pretreated with 250 mg oral potassium iodide 30 min before intravenous (IV) injection of 185 MBq $^{123}$I-mIBG ($T_{4.45}$). Fifteen minutes (early images) ($T_5$) and 4 h (late images) ($T_{8.45}$) after administration of $^{123}$I-mIBG, 10-min planar images were acquired.

Figure 2. Scheme of the study protocol. At $T_{0.15}$, $T_{2.15}$, and $T_{4.15}$, subjects received 500 mg AMPT (i.e. total of 1500 mg) or placebo. At $T_5$ (early) and $T_{8.45}$ (late) a 10 minute planar $^{123}$I-mIBG scintigraphy was performed.

Questionnaires
Blood sample

$^{123}$I-mIBG IV

Blood sample

Questionnaires

Blood sample

AMPT

AMPT

AMPT

Early H/M

Late H/M

$T_0$ $T_{0.15}$ $T_{2.15}$ $T_4$ $T_{4.15}$ $T_{4.45}$ $T_5$ $T_6$ $T_8$ $T_{8.45}$
All planar $^{123}$I-mIBG images were analysed by one experienced observer (D.O.V.) blinded to patient data. Heart-to-mediastinum (H/M) ratios were calculated from the $^{123}$I-mIBG images using a region-of-interest (ROI) over the heart and the upper part of the mediastinum (Figure 3).\textsuperscript{19} The H/M ratio was calculated by dividing the mean count density in the cardiac ROI by the mean count density in the mediastinal ROI.\textsuperscript{19} $^{123}$I-mIBG washout (WO) was calculated as followed:

$$WO = \left(\frac{\text{early H/M ratio} - \text{late H/M ratio}}{\text{early H/M ratio}}\right) \times 100$$

The H/M ratio reflects presynaptic uptake of $^{123}$I-mIBG. The early H/M ratio reflects predominantly the integrity of sympathetic nerve terminals (i.e. number of functioning nerve terminals and intact uptake-1 mechanism). The late H/M ratio offers predominantly information about neuronal function resulting from uptake, storage and release. The $^{123}$I-mIBG WO reflects predominantly neuronal integrity of sympathetic tone/adrenergic drive.

**Statistical Analysis**

All continuous variables are expressed as a mean ± standard deviation. After demonstrating a normal distribution of variables, between-group comparisons were performed by using independent-sample $t$-tests and paired $t$-tests. Statistical analysis concerning PRL levels at different time points were performed using repeated-measure ANOVA with a posthoc Bonferroni correction. A two-tailed probability value lower than 0.05 was selected as an indicator of statistical significance. Statistical analyses were performed with SPSS, release 22.0 for Windows (SPSS Inc., Chicago, IL, USA 2003).

**RESULTS**

**Demographic data**

Five subjects with 22q11.2DS and five age- and sex-matched controls, aged 20 – 39 years completed the protocol. The age (mean ± SD) of the subjects was 28.6 ± 4.8 and 28.0 ± 7.9 years, respectively. There were 3 males and 2 females in both groups.

**AMPT depletion**

Only three subjects (2 subjects with 22q11.2DS and 1 control) reported feeling tired after AMPT administration, which resolved in the hours after the last AMPT intake. No serious adverse events were observed.
Cardiac sympathetic activity in 22q11.2DS

Figure 3. Example of post processing planar $^{123}$I-mIBG images of a 22q11.2DS subject (late H/M ratio = 2.97). The positioning of the mediastinum ROI (M) is determined in relation to the lung apex, the lower boundary of the upper mediastinum, and the midline between the lungs. The manually drawn cardiac ROI (H) is placed over the myocardium including the left ventricular cavity.

Figure 4. Mean plasma prolactin (µg/L) levels following α-methyl-para-tyrosine (AMPT) administration in subjects with 22q11.2DS and controls. Both in 22q11.2DS and controls, administration of AMPT induced an increase in PRL, although this was only significant in the 22q11.2DS group.
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Table 1. \(^{123}\text{I}-\text{mIBG}\) scintigraphy results at baseline and after depletion with AMPT and the difference between baseline and after depletion with AMPT.

<table>
<thead>
<tr>
<th></th>
<th>22q11 DS (n = 5)</th>
<th>Controls (n = 5)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.67 ± 0.34</td>
<td>2.59 ± 0.33</td>
<td>0.652</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>2.80 ± 0.37</td>
<td>2.85 ± 0.32</td>
<td>0.465</td>
</tr>
<tr>
<td>(^{123}\text{I}-\text{mIBG WO})</td>
<td>-4.92 ± 2.82</td>
<td>-10.4 ± 7.29</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>AMPT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early H/M ratio</td>
<td>2.67 ± 0.25</td>
<td>2.50 ± 0.40</td>
<td>0.266</td>
</tr>
<tr>
<td>Late H/M ratio</td>
<td>2.93 ± 0.40</td>
<td>2.90 ± 0.33</td>
<td>0.641</td>
</tr>
<tr>
<td>(^{123}\text{I}-\text{mIBG WO})</td>
<td>-9.94 ± 7.11</td>
<td>-16.75 ± 7.66</td>
<td>0.786</td>
</tr>
<tr>
<td><strong>Difference baseline and AMPT</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Early H/M ratio</td>
<td>-0.00 ± 0.24</td>
<td>-0.09 ± 0.13</td>
<td>0.385</td>
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<tr>
<td>Late H/M ratio</td>
<td>0.14 ± 0.37</td>
<td>0.05 ± 0.23</td>
<td>0.463</td>
</tr>
<tr>
<td>(^{123}\text{I}-\text{mIBG WO})</td>
<td>-5.02 ± 5.35</td>
<td>-6.31 ± 5.48</td>
<td>0.970</td>
</tr>
</tbody>
</table>

**Peripheral markers of prolactin**

Baseline levels of PRL (T\(_0\)) were not significantly different between 22q11.2DS subjects (14.6 ± 3.0 μg/l) and controls (14.3 ± 3.2 μg/l). In both groups there was an increase of mean PRL levels 4h (T\(_4\)) after the first gift of AMPT (Figure 4). The levels of PRL subsequently decreased at T\(_6\). Compared with baseline there was no significant difference between both groups in mean PRL levels at T\(_4\) and T\(_6\). The change of PRL levels from baseline was only significant in the 22q11.2DS group.

**\(^{123}\text{I}-\text{mIBG scintigraphy}\)**

The results of the \(^{123}\text{I}-\text{mIBG}\) scintigraphy are shown in Table 1. At baseline and after depletion with AMPT there were no significant differences in early and late H/M ratio between the 22q11.2DS and the control group. However, there was a significant difference in \(^{123}\text{I}-\text{mIBG WO}\) at baseline between the 22q11.2DS and the control group (p = 0.027). AMPT did not significantly change early and late H/M ratio and \(^{123}\text{I}-\text{mIBG}\) WO compared to baseline in both groups. However, compared to baseline there was no significant difference in \(^{123}\text{I}-\text{mIBG WO}\) between the 22q11.2DS and the control group (p = 0.786).
DISCUSSION

This is the first study that investigated $^{123}$I-mIBG assessed cardiac sympathetic activity in adults with 22q11.2DS, a relatively common genetic condition associated with sudden unexpected death of unknown cause and impaired catecholamine turnover. In our study early and late H/M ratio in 22q11.2DS subjects were not different from healthy controls, and comparable with data published in other healthy (Japanese) subjects. In both groups late H/M ratio was higher compared to early H/M ratio. Consequently, $^{123}$I-mIBG WO was “negative” (i.e. indicative for increased $^{123}$I-mIBG myocardial uptake over time). The $^{123}$I-mIBG WO depends on the release, uptake and spillover of NE. In healthy subjects the myocardial NE release is on average > 20 times the myocardial spillover. In chronic heart failure (CHF) the presynaptic exocytosis of NE is increased and the uptake-1 mechanism, responsible for 90% of the NE re-uptake, is blocked resulting in increased spillover of NE. Therefore presynaptic levels of stored $^{123}$I-mIBG will decrease over time in CHF patients resulting in a lower late H/M ratio compared to early H/M ratio with consequently a “positive” $^{123}$I-mIBG WO. In healthy subjects the pre-synaptic NE release is limited and uptake-1 mechanism works properly resulting in accumulation of $^{123}$I-mIBG over time with higher late H/M ratio compared to early H/M ratio and consequently a “negative” $^{123}$I-mIBG WO.

Although there was no significant difference between 22q11.2DS subjects and healthy subjects in H/M ratio, the $^{123}$I-mIBG WO was significantly less negative in 22q11.2DS subjects compared to healthy subjects suggesting differences in adrenergic drive. However, the clinical effect of this difference in adrenergic drive is uncertain. As far as we know only increased $^{123}$I-mIBG WO (i.e. “positive” $^{123}$I-mIBG WO indicative for increased cardiac adrenergic drive) is associated with increased cardiac mortality and lethal arrhythmias. Therefore the results of our study suggest that cardiac sympathetic activity in adults with 22q11.2DS is probably not related to the incidence of unexpected death.

AMPT

In both groups PRL levels were increased shortly after the first administration of AMPT and subsequently decreased, indicative for the reversible tyrosine hydroxylase inhibition. However, in contrast to a previous study, there was no significant difference in PRL levels after administration of AMPT in the control group. In addition, there was no significant difference in PRL levels between 22q11.2DS subjects and controls. This could be explained by the relatively small sample size of the study.

Here we used, for the first time, the same AMPT paradigm used to assess changes in endogenous brain DA concentrations, to evaluate whether changes in endogenous peripheral NE concentrations may change the cardiac $^{123}$I-mIBG uptake. The present study showed a trend towards a higher late H/M ratio after acute depletion by AMPT.
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compared to baseline. This rise in late H/M ratio was more pronounced in 22q11.2DS compared to healthy subjects. This is in line with the hypothesis that levels of peripheral endogenous NE in 22q11.2DS are increased compared to healthy subjects.

In contrast to the significant effect of AMPT to peripheral NE markers, there was no significant effect at a group level on early and late H/M ratio and \( ^{123}\text{I}-\text{mIBG} \) WO. The lack of effect of AMPT on the scintigraphically assessed cardiac sympathetic activity may be explained by differences in NE spillover between different organs. In general the re-uptake of NE in the myocardium is very efficient and only contributes to 2-3% of the systemic NE spillover (i.e. plasma). Although there is a significant effect of AMPT on NE plasma levels, the myocardial NE re-uptake may be so efficient that the AMPT-induced changes cannot be visualized by myocardial \( ^{123}\text{I}-\text{mIBG} \) scintigraphy. Second, the used doses of AMPT may have been too low to induce a significant effect on the sympathetic activity. However, higher doses of AMPT may result in serious side-effects. Hypothetically a third explanation is that, to compensate for the decreased levels of NE, the uptake-1 mechanism is down regulated as in CHF, to increase the NE levels in the synaptic cleft and consequently diminish the effect of AMPT on \( ^{123}\text{I}-\text{mIBG} \) uptake. Finally, the timing between the start of administration of AMPT and the \( ^{123}\text{I}-\text{mIBG} \) scintigraphy might be suboptimal. In the present study a time window of 5 h was chosen based on the time window of \( ^{123}\text{I}-\text{IBZM} \) SPECT imaging and a significant decrease of NE (metabolites) levels 3 and 6 h after the start of administration of AMPT. It is speculative if a larger time window would have changed the outcomes of \( ^{123}\text{I}-\text{mIBG} \) scintigraphy. Nevertheless, the results tend to show that depletion with AMPT may be useful to investigate the cardiac sympathetic activity, but validations studies and larger trails are needed to demonstrate its effectiveness.

Our study has some limitations. First, the sample size of the study is relatively small and may have resulted in a limited statistical power. Second, although patients were randomly selected there is selection bias, as only those patients with 22q11.2 DS were enrolled who were physical and mental able to participate. Third, as a significant proportion of adults with 22q11.2DS have CHD and/or psychiatric disorders, and subjects with CHD and/or antipsychotic or psychostimulant medication were excluded, extrapolation of the current findings to the more general 22q11.2DS population is speculative. It may be of value to evaluate \( ^{123}\text{I}-\text{mIBG} \) uptake in these subgroups of 22q11.2DS patients as well.

In conclusion, this study for the first time suggests a normal \( ^{123}\text{I}-\text{mIBG} \) cardiac sympathetic activity in adults with 22q11.2DS. Although there is a small difference in adrenergic drive compared with healthy subjects, this most likely does not explain the increased unexpected death rate in the 22q11.2 DS population. Larger studies are necessary to confirm this hypothesis. The current data should be regarded as insightful but preliminary and extrapolation to the overall 22q11.2DS population should be done with great care.
REFERENCES


