**Physiological Data**

- The respiratory sinus arrhythmia (RSA), an indirect measurement of parasympathetic activity derived from R-R heart-rate variability, is substantially below age-matched norms, but increased a full standard deviation on 20 mg donepezil HCl (Aricept), an anticholinesterase inhibitor (P < 0.015).

- The pre-ejection period (PEP) is an indirect measure of sympathetic activity and a lower PEP is indicative of higher sympathetic tone. The PEP was normal, but increased above the normal range on donepezil HCl (Aricept) as expected.

<table>
<thead>
<tr>
<th>Table 2. Respiratory arrhythmia (RSA) and pre-ejection period (PEP) measurements of Patient 1 with CHAT mutation R628Q before and after treatment with donepezil</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSA (mmHg)</td>
</tr>
<tr>
<td>2.41</td>
</tr>
<tr>
<td>PEPS (ms)</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

**The CHAT Box**

**The data behind the case of clinical relevance**

CHAT encodes for choline-O-acetyltransferase, the enzyme that synthesizes acetylcholine. It is of special importance in the parasympathetic nervous system, acting as both the pre-synaptic and post-synaptic neurotransmitter.

**Why variants 340L>F and 510R>G in the CHAT gene are highly likely to be associated with clinical symptoms:**

1. Prevalence: *Uncommon* - 0.5% and 0.1% of the general population, respectively.
2. Evolutionary conservation: *Highly conserved* - 38/38 and 41/42 vertebrate species on the UCSC Genome Browser.
3. Computer Algorithms: *Predicted deleterious to protein function* – by MutationTaster, PolyPhen2, and SIFT.
4. Phenotypic diversity: *3 patients* in the 3 patients to substantiate reactions to drugs with known anti-cholinergic effects, in addition to "typical" mitochondrial disease manifestations.
5. Treatment Response: *Dramatic* - in 2 patients treated with anticholinergic esterase inhibitor donepezil (Aricept), including complete reversal of previous cognitive regression, better verbal and motor communication, and improvement in other autonomic features.
6. Physiological Data: *Very reduced parasympathetic activity* - statistically significant decrease in RSA in one patient to date, with significant improvement on donepezil HCl (Aricept) (P = 0.015).

**Conclusions**

- Extreme variable expressivity within a family is a well-documented phenomenon in mitochondrial disease, even when there is not an identified heteroplasmatic mutation.
- Oftentimes, as is the case in our three families, the proband is moderately to severely affected while several relatives are substantially less affected. This observation has prompted the frequent assumption that this variability might be due to "modifying genes".
- We now have the technical capability via NextGen sequencing to identify these modifying genes. This is of potential relevance to treatment, as the primary mutation may not highly amenable to therapy, but a modifying factor might be.
- CHAT is a good example of a modifying gene of mitochondrial disease in which its identification leads to specific therapy that demonstrates substantial efficacy. We were fortunate that there is an objective parameter to monitor and substantiate an observed clinical effect.
- Analysis of CHAT should be considered in cases of mitochondrial disease or dysfunction where there is an unusual response to medications with anticholinergic effects or substantial dystonia. Identification of a mutation would be helpful before anticholinergic medications are given.