



Development of targeted DNA-Chips for High Throughput Diagnosis of Neuromuscular Disorders

PROJECT SUMMARY

Inherited NeuroMuscular Disorders (NMDs) form a very large and heterogeneous group of genetic diseases that cause progressive degeneration of the muscles and/or motor nerves that control movement. The overall prevalence of NMDs is very difficult to evaluate, but one can estimate that, given the incidence of every different type, around **1 out of 1000 people** may have a disabling inherited neuromuscular disease, including Duchenne/Becker muscular dystrophies (DMD/BMD), limb girdle muscular dystrophies (LGMD), congenital muscular dystrophies (CMD), and hereditary motor-sensory neuropathies or Charcot-Marie-Tooth neuropathies (CMT).

The precise diagnosis of NMDs requires a conjunction of extensive clinical examination and targeted complementary tests: biological analyses, electromyography, imaging, and histological analysis of biopsies. Thus, a differential **molecular genotyping** by a **gene by gene** approach is required, which has until now been highly **complex, cost-effective and time consuming** (two weeks to one year). As a consequence, many patients remain **devoid** of genetic confirmation of their disease. To date, this proportion amounts to **30 to 40 %** of NMDs.

The aim of NMD-Chip is to **design, develop** and **validate** new sensitive high throughput DNA arrays to efficiently diagnose patients affected by NMDs. The tools (reliability from **95 to >99%**) originating from this project are designed to assess all known genes implied in a group of disease at one time, as well as to efficiently analyse chip data through optimised read-out bioinformatics tools, within **72hrs to one week**. Beside the development of these new high-throughput molecular diagnostics tools, NMD-Chip also fosters knowledge of NMDs by accelerating new disease causing mutations discovery, using a candidate gene approach.

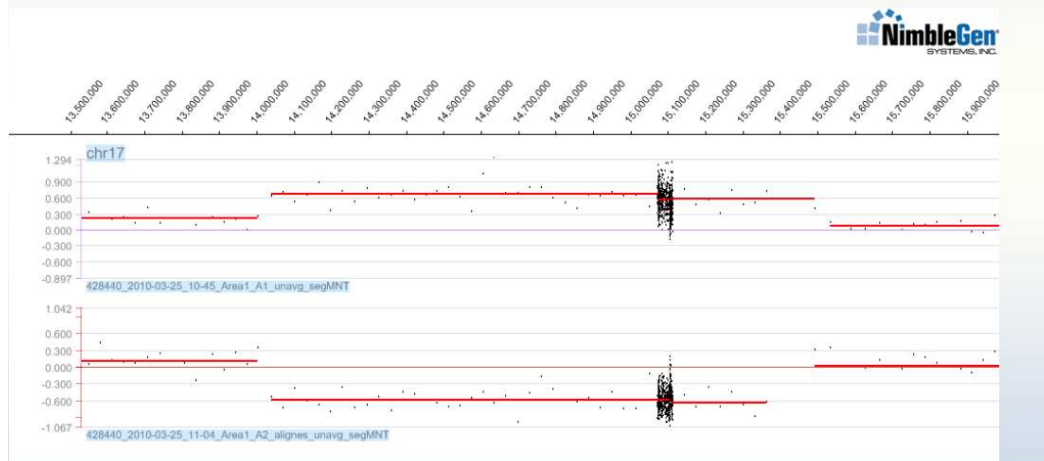
The **scientific strategy** was to design 4 types of chips, 2 for known NMD genes, and 2 for candidate genes. In each case, both CGH arrays to detect CNV (insertions or deletions), as well as Sequence Capture arrays for massive re-sequencing and point mutation detection, have been set up.

Today, at the end of the project, **the two first generations of CGH NMD-chips including all the known genes have been validated**, and are proposed to be spread to diagnostics workflow to progressively replace the current techniques. **The capture chips have been** compared with the "in-solution" capture tools that have emerged as an alternative solution during the two past years. This part of the work has not completely been achieved, and it is still unclear whether one approach is better than the other. The CGH chips have been validated on different previously characterised DNA with several deletions or insertions in LAMA2, DYSF, CAPN, DMD, PMP22, COL6A genes and others. One major problem remains after a first round of validation among the partners: for some particular

genes (for instance, EMD), the design must be improved since there is a lot of a variation in the results obtained so far. From this part of the project, **several uncharacterised patients have been provided with a molecular diagnosis, thus making the project a success.**

Array validation

• Duplications and deletions of the 1.5 Mb CMT1A region have been detected in **10/10** patients (Figure 1). In some cases, log₂ ratios were below the +0.2/-0.2 thresholds, but the number of duplicated/deleted probed does undoubtedly evokes the 1.5 Mb CMT1A duplication of the 1.5 Mb HNPP deletion.



Example of result obtained with the CMT CGH chip, in two CMT1A patients one presenting with the “classical” 1.5Mb PMP22 duplication, the other one with the mirror deletion.

For the research part of the project (**candidate gene exploration**), **the CGH chips have also been designed and validated.** The successes obtained with control DNA have convinced one partner to explore a cohort of 33 patients with an uncharacterised LGMD, with at the moment **one new CNV candidate** being further analysed. This CNV has been evidenced by qPCR and affects one of the candidate genes. As regards the **DNA captures** in the WP3, different approaches have been initialised, combining the commercial options (exome) with dedicated probes, from different providers. **This part of the work has been validated only with control known variations, which is already a successful outcome.**

In the meantime, **a large Reference Material database has been created** to collect the most relevant samples from each partner to be used as positive controls on the chips. From a bio-informatics point of view, several tools have been created to improve the chip design, to collect all the data, to analyse the pathogenicity of the mutations detected by the chips. A pipeline has been created to score and evaluate variants detected from the high-throughput sequencing data. In order to allow a rapid prediction of the pathogenicity of any SNP localized within an exon, by comparing them to the SNPs listed from the HG18 genome assembly, through our UMD-HTS system. Finally, the NGS-Viewer constitutes a complete pipeline to analyze NMD sequencing data from the raw data coming out of the sequencer to the final report that can be saved and exported to databases.

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Laboratory | Sample Material | View_sample_material_with_details | Experiment | Variant

Search for: Any field | Contains | Search | Show all | Details found: 6 | Page 1 of 1 | Records Per Page: 20

Add new | Inline Add | Export selected | Print selected

<input type="checkbox"/>	Internal ID	Characteristics	Purification	Quantity Available	Legal Status	Free Comments	Potentially Useful For CGH	Available	Exon Number	C DNA Access Number	HGVSNomenclature	Predicted Protein Change	Heterozygosity	Type	Detection Technique	Gene Name	Type1	Institution Name	Responsible Name
<input type="checkbox"/>	JAM1-test1	Cell line	Purification1	100	Free use within consortium	First test material entered in DB, as user 'jamel'	Unknown	Yes	1	NM_123	c.100A>T	p.30A>M	Heterozygous	SNP	Capillary sequencing	DYSF	Original		jamel
<input type="checkbox"/>	JAM1-test1	Cell line	Purification1	100	Free use within consortium	First test material entered in DB, as user 'jamel'	Unknown	Yes	3-5	NM_1111	delExon3-5	?	Homozygous	CNV	MLPA	NMD	Original		jamel
<input type="checkbox"/>	TEST22	White blood cells	Purification2	58	Free use within country of origin only	Another test material	Unknown	Yes	9	NM_76767	c.877T>A	p.200M>A	Mosaic	SNP	Capillary sequencing	RYR1	Original		jamel
<input type="checkbox"/>	JAM1-test1	Cell line	Purification1	100	Free use within consortium	First test material entered in DB, as user 'jamel'	Unknown	Yes	1	NM_123	c.100A>T	p.30A>M	Heterozygous	SNP	Capillary sequencing	DYSF	Validation		jamel
<input type="checkbox"/>	JAM1-test1	Cell line	Purification1	100	Free use within consortium	First test material entered in DB, as user 'jamel'	Unknown	Yes	3-5	NM_1111	delExon3-5	?	Homozygous	CNV	MLPA	NMD	Validation		jamel
<input type="checkbox"/>	47110815	Cell line	Purification1	234	Free use within consortium		Yes	Yes	1	NM_23675	c.180A>T	p.60P>C	Heterozygous	SNP	Capillary sequencing	RYR1	Original		jamel

Specific internal RM database user interface example

A logo, a website, an intranet website, and two different mailing lists have been designed to develop communication to the broader community as well as facilitating internal communication.

Several publications, oral or poster communications have emerged from this project. One important paper is under peer review that describes the complete workflow of the diagnostics from the patient to the result, as well as some results obtained, mainly during the validation steps. Several other articles are being written, directly related to NMD-chip. At least 5 external collaboration requests have arrived to the project coordinator that will certainly give rise to new project developments. Several countries have been in touch with the coordinator to follow the chip dissemination, and they are interested in commercialising and using the chips. Two patents protect the whole workflow of the “muscular diseases” and CMT diagnostics.

Finally, even if too few experiments have been achieved with capture technologies, all the aspects of the project have been explored and have to be considered as successful. Several proofs of concept have been obtained, some patients have been provided with a diagnosis even though the chips are not commercialised yet, new mutations have been characterised, and at least one new candidate gene is under exploration. Moreover, people have been able to work together, and all the different aspects of the project (the different committees, the regular meetings, the contractual release of documents) has worked as well as possible. As a consequence, all milestones have been achieved (but one related to a joint meeting with TREAT-NMD), and all deliverables and reports have been released during the project course.