Minutes DITF Ithaca Break Out Session Annual Meeting 2020 Barcelona

Date: March 5, 2020

DITF present in Barcelona: Lisenka Vissers (chair; RUMC), Elke de Boer (minutes; RUMC), Elisa Benetti (Siena), Anne Sophie Denomme-Pichon (CHU Dijon), Antonio Vitobello (CHU Dijon), Alain Verloes (INSERM), Olaf Riess (EKUT), Marco Tartaglia (IRCCS), Estrella Lopez Martin (UDP Spain), Vincenzo Nigro (Telethon UDP)

DATF in Barcelona: Christian Gilissen (RUMC), Karolis Sablauskas (RUMC), Wouter Steyaert (RUMC), Morris Swertz (UMCG), Peter Bram t Hoen (RUMC); Carles Garcia (CNAG); Leslie Matalonga Borrel (CNAG)

DITF attendance via Zoom: Jill Clayton-Smith (MUH), Adam Jackson (MUH), Alessandra Renieri (Siena); Lukas Ryba (CUNI)

SOLVE-RD project management: Kornelia Ellwanger (monitor Zoom attendance)

Apologies: Aurélien Trimouille (CHU Bordeaux), Tobias Haack (EKUT), Laurence Faivre (CHU Dijon), Birte Zurek (EKUT), Sanja Hermanns (EKUT), Caroline Rooryck-Thambo (CHU Bordeaux), Anna Lindstrand (KI), Francesca Clementina Radio (IRCCS), Holm Graessner (EKUT), Georgio Casari (Telethon UDP), Siddharth Banka (MUH), Alexander Hoischen (RUMC), Manuel Posada (UDP Spain), Milan Macek (CUNI), Ann Lindgren (KI),

Agenda and goals of the meeting

- Update unsolved cohort – numbers and use cases
- Patient selection for WGS ERN-specific cohort
- Patient selection for omics unsolvable syndromes
- Use case definition: revisiting the old ones, and making new ones
- AOB

General remarks from WP2

- **<u>Next data freezes for data-processing of the unsolved cohort</u>**
  2nd: September 2020
  3rd: March 2021

- **On the novel omics**
  25% less SR-WGS available for ERN-specific cohorts and ultra-rare cohort, because of a smaller price drop for SR-WGS than was anticipated.

Q Alain Verloes: how do we solve the issues with consent? LV: how to ship samples if you are not in ERN or Solve-RD? This relates to the consent issues. It is possible to include patients from other centers/countries in the Nijmegen biobank. Carles Garcia: Also public interest as reason for inclusion (in which patient is informed for use of data and where it will be used), instead of consent for study. Communication is enough. LV: GDPR includes a clause that states if interest for public health or research, it is possible to do research without consent on anonymized. However, this is an exception in law. For our cohort, it would be better to go for having consent of patient.

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The Unsolved cohort

Within SOLVE-RD, ERN Ithaca promised ~5000 unsolved exomes/genomes.
Q: Where do we stand today, and are we able to reach the numbers promised?

Overview of samples per partner:

ITHACA partner	Difference between what currently is uploaded and what was promised (datasets)	Expected additional upload (individuals) at data freeze 3 (March 31, 2021)	Comments
Nijmegen	369	1500	500 trios
Dijon	297	297	297 for sure, maybe more (including genomes)
Manchester	86	100	
Tubingen	250	250	
Prague	-	?	
Siena	669	1350	450 trios (1350 individuals), maybe more
Rome	41	40-100	Between 40-100 probands
Stockholm	150	?	Not present, will be confirmed later
Bordeaux	37	?	Not present, will be confirmed later
UDP Spain	10	Max 150	Have 50 trios with exome analysis currently ongoing, so maximum 50 trios, but presumably less. Possibly few genomes as well
Telethon Italy		?	Consent issues
Paris		200	200 (not sure index/or trios)

Q of Alain Verloes: new HCPs entering ITHACA, how to include these? LV: If >100 exomes uploaded, will become partner.

Conclusion: We are reaching the numbers we have promised at the start of the project, which is great news!

Note: The project does not discriminate whether or not the ~5000 data sets refer to affected or unaffected individuals. It is Ithaca's ambition to get close as possible to the 5000 affected individuals, but we will count unaffected parents as 'separate data set' in all overviews listing 'numbers of datasets'.

Action points:

- Upload new data prior to data freeze 2. Ideally this should not wait until the last week prior to the deadline (all)
- SOLVE-RD participants who have not yet confirmed their numbers, please confirm the numbers you expect to upload for data freeze 2 and 3 to LV
- Identify new HCPs in the ERN with large WES data sets for inclusion of their unsolved patients in SOLVE-RD (LV and Alain Verloes)

Feedback on use case 1: variant calling and annotation
See slides for details on numbers.

Efforts on re-analysis of variants by working group 1, has resulted in diagnosis of 1.7-3.4%.

Q Antonio Vitobello: If this could have been done before, the numbers could have been better, as centers have solved some of the cases in the meantime. LV: This also applied to Nijmegen. In the future, there will be shorter time frames between upload and feedback of data-analysis. Reasons for the first data-analysis to take longer than expected are mainly logistic reasons.

Discussion point: How to get information on solved cases back to the system and how to get this information back to the patient? Q Morris Swertz: how can we arrange this? Technical solution?
Q Antonio Vitobello: worked on a gene and tagged few variants as likely pathogenic, is there an automatic way to get this information to the system (PhenoTips)? Carles Garcia: Currently it is not yet available to do this in bulk in the RD-connect platform, the team is working on this, not yet sure when this will be ready, but expected in the near months.

Discussion point: There would need to be a technical solution for getting access to all files, as some might want to go back to BAMs.

Marco Tartaglia: Why were these variants in WG1 reanalysis not found before? That would be very useful to improve/compare pipelines. LV: several reasons: new information in literature on new genes, some were already solved in the meantime, others had to do with remapping and recalling. GENTURIS example: 2 cases had the same variant in same gene, in one patient it was called/annotated by WG1 reanalysis, but not in the other. No other differences that could explain the difference. WG1 will likely ask these questions to the DITFs soon.
Q Antonio Vitobello: Leslies approach did not take into account truncating variants in genes with a high pLI.

Marco Tartaglia: Variants in recessive genes, look for second hit in genomes in these genes? LV maybe we can re-allocate some of the genomes from ERN-specific cohorts/ultra-rare for this. Similar analysis have been successful for other rare disorders.

Feedback on relatedness:
Cohort approximately 4% consanguinity. Good confirmation that our cohort is a representative of unsolved ITHACA patients, as similar numbers are reported in literature.

Meta-analysis:
Christian Gilissen: Delays in meta-analysis, as all data has to be transferred to Nijmegen. Downloading of BAMs started Jan 10, currently ongoing. Connection is poor/disruptive at the EGA location. An update of EGA is planned for in March, at which time the server will not be available.

The BAMs need to be available in Nijmegen to allow de novo calling required for our meta-analyses. It is expected to have all the BAM-files in April (although a bit unsure given the poor connection). It is possible to have results approximately 2 months after all files have been downloading to Nijmegen.

LV: As this dataset of ID-cohort is relatively small (1500) compared to some cohorts in literature, it is unlikely that it will provides us with lots of new statistically enriched genes. But it will give us new genes, e.g. if 2 patients have a de novo variant in the same gene.

Alain Verloes: Last year we had discussion to extend meta-analysis to other cohorts (e.g. Care4Rare). LV: Will reach out again, but have discussed with Kym Boycott (Canada) and Gareth Baynam (Australia) before. Reason for postponing this was that systems/logistics are not yet ready to also have data of other cohorts.

Antonio Vitobello: Analysis of oncogenes (incidental findings). Are we going to do overall analysis including all ERNs, and what will be done with oncogenes? LV: Karolis (PhD students) did overlap HPO analysis on ERNs, and the ERNs show considerable overlaps.

Antonio Vitobello: What about oligogenic inheritance? LV: I don't think this has been envisioned in the project within Ithaca, but the datasets gathered in SOLVE-RD provide an excellent start for these hypothesis. 1600 likely pathogenic variants (65% of all indexes, mostly inherited from an unaffected parent) in known ID genes in our cohort alone is already intriguing – perhaps this is a signal worthwhile investigating? It would be a good idea to also do the ITHACA gene list in the other ERNs, to see if this just comes with the gene list, or that we have an enrichment in ITHACA for certain genes – either way: we should come up with use case, and likely Genturis has use cases defined that are along the same lines as they plan for burden analysis. Action point for DITF leads to follow-up on and discuss.

Conclusions on unsolved cohort: Many analyses are still ongoing but our first results look promising. We have achieved a diagnosis for the first unsolved patients which is a great milestone for the project!

Action points:

- Monitor data processing for meta-analysis (LV in DITF-DATF calls)
- Discuss possibilities for burden analysis with other DITF leads (LV in DITFlead call)
- Reach out to other projects worldwide for participation in meta-analysis (LV)

ERN-specific cohorts

Goal: to decide which and how many patients to sequence with SR-WGS

Overview:

Disease	***Number of individuals with samples available***	***Number of total eligible individuals***	***Notes***
Rett-like patients	*33*	*33*	*These are trios, and one familial (2 affected siblings)*
Poland-Moebius	*7*	*10*	*These are indexes*
Goldenhar	*30*	*100*	*1 trio with DNA available, maybe 2-3 additional cases Sienna, Rome 20-29 cases, Bordeaux large cohort appr 200 cases. Let's aim for 100 genomes*
Wildervanck	*18*	*19*	*1 trio, 4 solo exomes negative and 1 case with candidate in exome but probably does not explain whole phenotype. DNA of 6 trios available.*
Rasopathies	*100*	*100*	*approximately 30 trios from Rome*

Rett-like syndrome (Elisa Benetti):
33 samples, 9 trios and 1 familial cases with 2 affected siblings, negative exomes.
Olaf Riess: How to define Rett-like. Did patients have regression?
LV: And are these already re-analysed in GPAP? EB: Not yet, currently being uploaded to GPAP, only recently performed exomes, analysis in Siena.
Olaf Riess: in one of the pedigrees 5 affected individuals, did you see them all? Alessandra/Margarita (zoom): Some of those cases are ID, others are epilepsy (coloured differently, but problem with projection on screen). The 2 boys have different phenotypes. Boys not related, so probably different disease. LV: Is there DNA available for segregation? EB: Now only trio, might be recontacted.
Conclusion: All 33 samples approved for WGS upon a negative WES result.

Moebius-Poland-like syndrome (Elisa Benetti):
10 patients, 7 DNA available, others resampling.
Alain Verloes: Are we collecting isolated Poland cases/Moebius cases, or preferably patients with features of both? Now we have all three, mostly isolated, but also one case with both from Dijon.
LV: What would be the strategy, trios, or indexes? Overlap? How to analyze? Is parental DNA available?
Marco Tartaglia: Would prefer to do trios. And it should be a standardized cohort with very consistent clinical features.
Conclusion: trios prerequisite.
One familial case, proband and affected mother, both DNA available.
Olaf Riess: skin biopsies? Elisa: case from Dijon skin biopsy is planned, not always feasible e.g. because of young age.
Marco Tartaglia: could be secondary to vascular abnormality. We should really think about an approach/hypothesis, otherwise we will not find anything as it is presumably genetically complex.
Alain Verloes: Why it is only the upper part of the body (chest/arms and brainstem but never lower limbs)
LV: should we postpone the genomes for Poland-Moebius then, if we not yet have a clear hypothesis? Olaf Riess: don't wait, do genomes. LV: consider to do deeper genomes if mosaicism is expected??

Conclusion: All *families* presented (affecteds and unaffecteds) are eligible for WGS (if WES is negative).

Goldenhar syndrome (Elisa Benetti + Rome):
1 trio in Siena with DNA available, 2 additional cases to recontact. Rome 20-29 cases, Bordeaux large cohort of maybe 200 cases.
Conclusion: Aim for ~100 genome (e.g. 30-35 trios).

Rasopathies (Marco Tartaglia)
30 cases in Rome, WES ongoing for 10 of these. In collaboration with Maagdenburg and Paris.
Conclusion: WES negative trios are eligible for WGS (e.g. 30-35 trios).

Wildervanck (Anne-Sophie Denommé-Pichon)
5 candidate patients, but none of them has the typical features (1 trio, 4 solo). DNA available for all the trios. One extra patient has a candidate in exome but presumably does not explain complete phenotype. Include as well.
Conclusion: 6 trios, 18 samples, all eligible for WGS after negative WES.

General conclusions ERN-specific cohort:

- All samples/families presented in Barcelona are eligible for WGS, upon a negative WES result.
- Definition of negative WES: negative analysis locally, and a negative analysis after upload in SOLVE-RD reanalysis (as performed in use case 1)

Action points:

- Upload of patients (and family members) with HPO and negative exomes in SOLVE-RD environment. Upload of data will be coordinated by the collaborators listed with the disorders in these minutes.
- Start shipment of samples to Nijmegen for further processing for WGS in SOLVE-RD using protocols which will be confirmed by Alexander Hoischen (WP2) soon after the AM2020.

Unsolvable Syndromes

Goal: to decide which patients to include in multi-omics

Overview

Disease	***Number of individuals with samples available***	***Number of total eligible individuals (pt and if available parents)***	***Notes***
Gomez Lopez Hernandez	*13*	*40*	*3 trios, 1 quad - lot of other patients-parents, of which some would be more eligible for replication*
Hallermann-Streiff	*9*	*34*	*3 trios*
Aicardi	*12*	*30*	*4 trios, also includes Siena with VUS*
Pai	*12*	*18*	*Additional patients with duo or solo analysis?*
OAFNS (oculo-auriculo-fronto-nasal syndrome)	*3*	*3*	*Additional patients with duo or solo analysis?*

Pai and OAFNS – same spectrum (Antonio Vitobello/Anne-Sophie Denommé-Pichon)

See slides for plans and patients.
Q Also patients with duo or solo. What should we do with those cases?
LV: not for initial discovery cohort – the OMICS analysis should not be hampered by 'missing data from relevant family members in the interpretation fase'. But, please do collect these samples for the replication cohorts.

Q Marco Tartaglia: Do all omics at the same time or do first one test and then the other?
LV: these are unsolvable for a reason. Solve-RD provides us to do everything at once. Even if 'one omic' provides 'the molecular answer', the other omics would still be interesting too are these may provide valuable information from a scientific point of view, for instance in additional functional read-outs.

Q Jill Clayton-Smith: Is there overlap with heminasal aplasia with prolateral probosus? Should we include?
Yes, include, especially in the replication cohort be as inconclusive as possible.

Aicardi (Elke de Boer)

See slides for details.
Typical and/or 'typical+' patients. 10 trios ideally for further studies. For three Nijmegen trios all samples for all omics are available. Patients approved as typical Aicardi.
Also trios from Siena. One trio tested with mono-allelic POMT1 mutation (pat inherited, high CADD score, VUS, but phenotype overlaps with Aicardi). For this case, still inclusion in Aicardi cohort for multiple omics approach.
Additional case from Dijon; Many omics already done (Exome, WGS, Transcriptome).
Also one trio from Montpellier.

Note Alain Verloes: Check ophthalmologic reports for Sienna patient 2, if available, as the eye abnormality is a typical hallmark signature for Aicardi syndrome.

Conclusion: move forward with the four trios available and add the remaining samples when they become available through resampling.
Action point: Upload sample with available omics in SOLVE-RD (Dijon) to allow for further analysis in the project.

Gomez Lopez Hernandez syndrome (Elke de Boer)
See slides for patient details.
Nijmegen: Many samples, however, none have the classical triad.
Nijmegen 1 and patient 2: approved
Nijmegen Sisters (patient 3a and 3b): approved
Nijmegen 4: replication cohort (has VUS in unknown gene): approved
Nijmegen: three trios and one quad: all samples available.

Manchester 1: no RES → replication cohort
Manchester 2: approved
Dijon 1: sampling planned (VUS in FGFR2).
Dijon 2: VACTERL considered before. Samples planned. approved
Dijon 3: RES but normal cognition. approved
Ithaca inclusion pending: Essen 1: LR-WGS (low coverage already performed)
Essen 2: also EP300 variant, but not explaining full phenotype
UDP Spain 1: no ID/DD, RES+: proband, mother, daughter
UDP Spain 2: resampling not possible, patient deceased

Decision: no issue with not fulfilling triad; but RES essential. Those without RES in replication cohort.
Exception: sisters (Nijmegen 3a/3b) of quad as these may be very helpful in gaining biological insights into GLH.

Hallermann-Streiff (Elke de Boer)
Nijmegen 1: all samples available (Clinically: Werner syndrome considered, but variants in known genes excluded). Approved
Amiens 1: approved
Nantes 1: approved; sample taken soon
Leuven 1: approved; samples available
Leuven 2,3,4: re-sampling to be done
Pécs 1: to be decided
Lille 1-2: recontact clinician
Tours 1-2

Conclusion: 3 good trios ready, resamples on the others to be taken place soon. Continue OMICS for those with samples available and add patients for whom re-sampling needs to take place at a later stage.

Note Alain Verloes: HSS not a typical syndrome, but a spectrum. Individual clinical phenotypes not typical/specific. One could also define them as progeria with cataracts. Expect not 'one' typical gene, but perhaps novel mechanisms of known genes (for instance those in the progeria spectrum)

General conclusions Unsolvable cohort:

- For all unsolvable syndromes, we have samples already collected for all omics. We will move forward with these samples and add novel patients later (upon availability of Omics available and/or for the replication cohorts)

Action points:

- Upload of patients (and family members) with HPO and exomes/genomes (if available) in SOLVE-RD environment. Upload of data will be coordinated by the collaborators listed with the syndromes in these minutes.
- Start shipment of samples to Nijmegen for further processing for WGS in SOLVE-RD using protocols which will be confirmed by Alexander Hoischen (WP2) soon after the AM2020.

Use case definition

No time available for detailed discussions. When the unsolved cohorts were discussed, many hypotheses were put forwards. We need to rephrase these into use cases. This will be put on the agenda for the next DITF call (26th of March 2020).

AOB:

Ultra-rare Cohort

- SOLVE-RD has reached to all HCPs (at the 2019 Board Meeting in Dusseldorf) to submit an ultra-rare case (as trio/family) for WGS. So far, only very few cases have been registered.

Action points:

- Reach out to ERNs again in (for instance) the newsletter with details explanation on how to submit cases. (LV + Alain Verloes)

Next DITF CALL

MARCH 26,2020, 14-15h