Final report of CATEGORY 1A: Visit by the applicant to another laboratory

Awardee Name: Walaa Allam
Home institution: Center for Genomics, Zewail City of Science and Technology
Visiting institution: University Medical Center Schleswig-Holstein, Campus Kiel
PI of host laboratory: Gregor Kuhlenbaeumer

This fellowship aimed at learning whole exome sequencing (WES) data analysis and strategies for variant identification. We focused on studying an Egyptian family who is suffering a rare genetic disorder that despite extensive clinical investigation remains undiagnosed. This family consists of seven siblings born to distantly related (sixth-degree consanguineous) healthy parents. The siblings are four females aged 21, 19, 18 and 15 years and three males aged 11, 10 and 3 years (Figure 1). The first female sib (21-year-old), the second female sib (19-year-old) and the sixth male sib (10-year-old) were affected by the disease. Family history of both parents' families was positive for multiple consanguineous marriages and multiple similar cases showing features of neurological and endocrinal dysfunction.

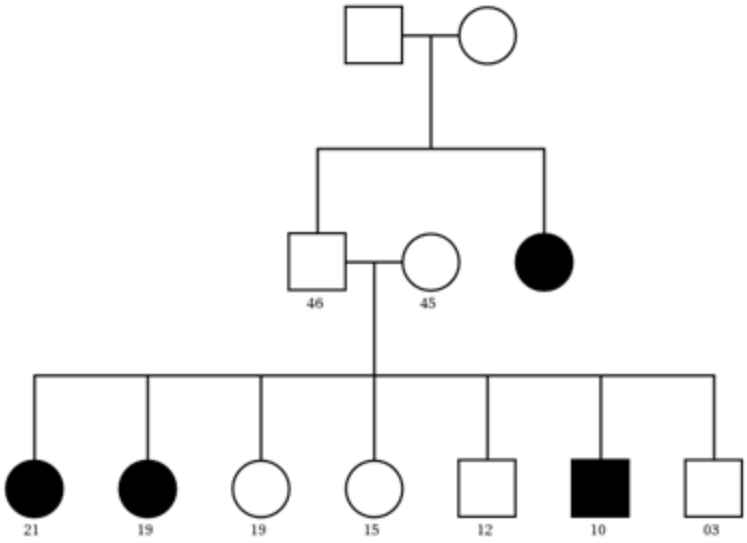

Figure 1.Family consists of seven siblings (four females and three males) are born of distantly related healthy parents. Two girls and one boy were affected by the disease.

The Radiological evaluation revealed supratentorial white matter high intensity areas in T2-weighted images which appeared iso/hyperintense in T1- weighted images compatible with hypomylination associated with typical sparing of the anterolateral aspects of thalami, globus pallidi, internal capsule and optic radiation. In addition, there was thinning of corpus callosum and mild cerebellar atrophy in the 6th brother (more pronounced at the vermis) compared with the cerebellum of aged-matched controls. All other members of the family had normal MRIs of the brain. The Laboratory evaluation revealed elevated Alp, LDH & Phosphate levels in all affected sibs, elevated triglycerides in the 1st and 2nd sisters, elevated HDL in the affected brother. Also, HDL was reduced in the 2nd sister.Both clinical, radiological and laboratory examinations indicated that the children are affected with 4H leukodystrophy which that leads to abnormal

development or destruction of the white matter (myelin sheath) of the brain, hypogonadotropic hypogonadism and hypodontia.

4H leukodystrophy is caused by mutations in one of three genes: POL3RA, POL3RB and POLR1C. We used whole exome sequencing to look for mutations in any of those genes. In Zewail City of Science and Technology we collected peripheral blood samples from the affected siblings after obtaining informed consent. Genomic DNA was extracted using standard methods and WES was carried out using paired-end sequencing on the Illumina NextSeq 500 instrument generating 100-bp reads. The output reads from the Illumina NextSeq 500 were mapped to the reference haploid human-genome sequence (Genome Reference Consortium human genome build 37; human genome 19) with the use of the eland (Illumina, Inc) generating per-sequencing-lane bamfiles. Fastq files derived from per-sequencing-lane bamfiles were re-aligned to the reference sequence using the Novoalign program. The generated Fastq were annotated by the annoVar in UKSH and then the R-tool was used for analyzing the data and filtration of the variants. Also, the UKSH WES analysis pipeline was used for confirmation of the accuracy of manual analysis. The analysis revealed that the three affected sibilings are suffering a mutation in POL3RB gene (chr12:106848414, A > G), however this mutation was reported to be "likely benign" by two independent submissions on the Cinivar data base, however we are still considering a role for this variant in developing the pheotyoe. At the same time, we have filtered out variants (heterozygous, >0.05% population frequency, X-linked, inaccurate alignment, etc.) and we are considering another five genetic variants (SERPINA1, TP53I3, TOR3A, PLCL2, RDH8) for a potential role in the this disease.

Outcomes and benefits of the fellowship:

1. The student gained in depth experience on quantification and quality assessment of samples and sequencing libraries which will contribute to a cost effective use of sequencing libraries/reagents in her home institute.
2. Hands on training on library preparations of genomic DNA samples.
3. Understanding whole exome sequencing data analysis approaches and how it is performed.
    a. using R-language for data trimming
    b. Variants and gene prioritization strategies for genetic diagnosis of diseases, based on variant allele frequencies, genotype frequencies, inheritance models, family history and patient instead of simply identifying potentially damaging variants.
4. Initially diagnosing an Egyptian family suffering a rare genetic disorder to be infected with a 4H mutation. This pathogenic burden of the mutation will be assessed by further mechanistic studies.
5. Establishing collaboration between Zewail City and Universitätsklinikum Schleswig-Holstein (UKSH); both institutions are currently working on another project for diagnosis of genetic disorders in Egyptian consanguineous children.
6. Contributing to the applicant's Ph.D. progress where the research results from this fellowship will be presented in the thesis and a research article is expected to be published based on this work within six months.

7. Establishing WES analysis workflow in Zewail city, will be used for analysis of Egyptian patients samples, will aid to patients clinical diagnosis and improving their life quality.

Financial report:

Item	Cost ($)
Airfare (Cairo airport to Berlin)	600
Local travel	100
Visa fees	150
Health insurance	150
Accommodation (rent, utilities, transportation, etc.)	2300
Total	3500