SCD Screening: Methodology, Laboratory Practice, and Cases

Georgi Manukjan[1], Ute Holtkamp[1], Ella Janzen[1], Thomas Neisse[1] and Nils Janzen[1]

[1]Screening-Labor Hannover, Am Steinweg 11A, 30952 Ronnenberg, Germany

In Germany, yearly about 200 children are born with sickle cell disease (SCD), typically diagnosed between the ages of three months and five years.[1] SCD is associated with severe complications such as recurrent pain episodes, infections, acute chest syndrome, and stroke.[2] In September 2021, SCD screening was incorporated into the national newborn screening program.[3] Early detection is highly beneficial, as it allows for prompt initiation of treatment and provides an opportunity to educate parents at an early stage.

In our laboratory, screening for SCD is performed using a molecular genetic melting curve analysis approach. DNA is extracted from a 3.2 mm dried blood spot punch and amplified via multiplex qPCR, specifically targeting the hotspot mutation c.20A>T in the hemoglobin subunit beta (*HBB*) gene. The amplified products are then subjected to stepwise denaturation with simultaneous fluorescence measurement. This generates characteristic melting profiles that distinguish between wild-type samples and heterozygous or homozygous variant carriers. Samples displaying deviations from the homozygous wild-type profile are subsequently confirmed using high-performance liquid chromatography (HPLC) as a second-tier analysis method. The benefits of this approach are time effectiveness and unambiguous assignment. For instance, false negative findings in case of erythrocyte transfusion could be neglected as well as dependence on timely blood draw.

From over 735,000 screened newborns since the onset of SCD screening until today, about 4,200 were positive in our first tier approach, meaning an approximate minor allele frequency (MAF) of 0.0029 for *HBB:* c.20A>T. From these, 148 suspicious findings were reported by second tier analysis, resulting in a prevalence of 0.20 per 1,000 births. Both, MAF and prevalence align well with local population data. Finally confirmed, our cases could be classified into 85 “classical” HbS/HbS homozygous SCDs, 15 HbS/HbC compound heterozygous, 30 compound heterozygous for HbS and a beta-thalassemia variant, and 7 homozygous beta-thalassemias. Two HbS/HbS cases are also heterozygous alpha-thalassemia carriers. Furthermore, one HbG-Philadelphia/HbS and one Hb Handsworth/HbS compound heterozygous individual were identified, respectively. Yet, 7 cases remain unconfirmed.

Referrals to hematology centers proceed using the contact information provided on the GPOH Konsortium website. First, the centers are contacted by email; once they have given approval, centers directly contact parents and schedule appointments for confirmatory diagnostic tests. This has proven as efficient process in terms of compliance and limits parental concerns.

References

1. *IQWiG Final Report [S18-01]: Screening for sickle cell disease in newborns; 09/2019.*
2. *AWMF Guideline 025/016: Sickle Cell Disease; 07/2020.*
3. *Final Report of the Federal Joint Committee (Gemeinsamer Bundesausschuss; G-BA) on the Children's Guideline: Screening for Sickle Cell Disease in Newborns; 03/2021.*