

WEB ANNEXURE 1

DETAILS OF SUBJECT EVALUATION

<i>Evaluation</i>	<i>Details</i>
Historical	<ul style="list-style-type: none"> Clinical symptomatology (abdominal pain, satiety, vomiting, dyspepsia, jaundice, drug intake etc). Family history of metabolic diseases
Physical	<ul style="list-style-type: none"> Anthropometric parameters (all measured in triplicate and average taken) <ul style="list-style-type: none"> Height and weight were measured using portable stadiometer and weighing scale to the nearest 1 mm and 100 grams, respectively. Body mass index (BMI, as weight in kilograms/height in metres²) percentile according to age and gender. Waist Circumference (WC) measured in standing position with tape applied along a horizontal line just above the uppermost lateral border of the right ilium to nearest 1 mm. Hip circumference (HC) measured in centimeters (cms) at the largest diameter of the hips. Waist-Hip ratio was calculated as a ratio of WC and HC. Overweight diagnosed if BMI between the 85th and <95th and obese if it ≥95th percentile for age and gender. For adults (≥18 years age), overweight and obese defined as BMI ≥23 and ≥25 kg/m² respectively. Abdominal obesity- (a) WC ≥90 cm (males)/≥80 cm (females) for adolescents aged ≥16 yr or adults, (b) for children aged 10 - <16 years, WC ≥90th percentile or adult cut off if lower and (c) for children <10 years age, ≥90th percentile. WC percentiles based on published Indian data. Blood pressure (BP) measured (in millimetres of mercury or mm Hg) in sitting position, with cuff at heart level using an aneroid sphygmomanometer with appropriate sized cuff (cuff bladder encircled at least 80% of the mid-upper arm) after 5 minutes of rest. <ul style="list-style-type: none"> § Systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) <90th percentile were reported as normal. SBP and/or DBP between 90th and 95th percentile or BP ≥120/80 at any age was defined as prehypertension, Stage 1 HTN as >95th but <99th percentile plus 5 mmHg and stage 2 HTN as > 99th percentile + 5 mmHg. Acanthosis nigricans defined as increased pigmentation of skin (darkening) and thickening (hyperkeratosis) of the skin, mostly in the nape of neck, the axilla and/or groin.
Laboratory	<ul style="list-style-type: none"> After an overnight fast- aspartate aminotransferase and alanine aminotransferase (standard automated kinetic enzymatic assay), lipid profile (total cholesterol, high-density lipoprotein cholesterol or HDL, and triglycerides or TG, using enzymatic-calorimetric test), serum uric acid and fasting plasma glucose (glucose oxidase method) and insulin levels (immunoenzymometric assay). Homeostasis model assessment 1- of IR (HOMA1-IR) calculated as fasting insulin (mIU/l) × fasting glucose (mg %)/405. HOMA1-IR >2.5 was taken as an evidence of Insulin Resistance (IR). The HOMA2-IR index was obtained by the program HOMA Calculator v2.2.2. The quantitative insulin sensitivity check index (QUICKI) calculated as 1/[log (Insulin ¼U/mL) + log (Glucose mg/dL)]. Metabolic Syndrome (MS) defined as per standard criteria and divided according to the age. MS definition required presence of abdominal obesity (as per WC) plus ≥2 or more of other parameters (elevated TG, low HDL, high BP, and hyperglycemia). In those patients fulfilling hyperglycemia definition (FBS ≥100 mg%), an oral glucose tolerance test (OGTT) (2-hour plasma glucose testing after 1.75 g/kg, maximum 75 g, glucose load dissolved in water) and fasting glycosylated hemoglobin (HbA1c) testing was done. Diabetes and pre-diabetes defined as per standard criteria. Ultrasonography of abdomen was performed by trained operators using a scanner with a 3.5-MHz transducer. Standard grading system used. Transient Elastography was done using Fibroscan apparatus (Echosens, Paris, France) by trained operators with experience of > 500 examinations. LSM and CAP were calculated based on principles and examination described previously. The LSM (as kilo pascals or K Pa) was considered reliable only if 10 successful acquisitions were obtained and success rate was >60% and, interquartile range was <30%. The CAP was measured on validated measurements as per LSM criteria with the final value as the median of

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<i>Evaluation</i>	<i>Details</i>
	individual ten CAP values (expressed as dB/m). As per manufacturer's guidelines, CAP measurements were done using the 'M' probe in the study subjects.
Liver biopsy	Percutaneous liver biopsy performed using the 18G trucut biopsy needle in those children in NAFLD group in whom there was persistent elevation of transaminases (ALT > 40 IU/L) despite atleast 3 months of diet control. NAFLD activity score (NAS) was used for evaluation activity in cases of NAFLD where a score ≥ 5 suggested NASH.

Details of *PNPLA3* Polymorphism Testing

For *PNPLA3* analysis, genomic DNA (gDNA) was extracted from peripheral blood by the phenol-chloroform method. The *PNPLA3* rs738409 C>G SNP was identified following the polymerase chain reaction (PCR) using the forward primer 5'-TGGGCCTGAAGTCCGAGGGT-3' and reverse primer the 5'-CCGACACCAGTGGCCTGCAG-3'.